Review

Oxygenomics in environmental stress

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Environmental stressors such as chemicals and physical agents induce various oxidative stresses and affect human health. To elucidate their underlying mechanisms, etiology and risk, analyses of gene expression signatures in environmental stress-induced human diseases, including neuronal disorders, cancer and diabetes, are crucially important. Recent studies have clarified oxidative stress-induced signaling pathways in human and experimental animals. These pathways are classifiable into several categories: reactive oxygen species (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. This review describes the gene expression signatures through which environmental stressors induce oxidative stress and regulate signal transduction pathways in rodent and human tissues.

Keywords: Oxygenomics, environmental stress, gene expression signatures, signal transduction pathways

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 human diseases. Environmental stress is a key contributor to human disease. Myriad substances such as metals, particulate materials, smoke, pesticides, and physical agents are environmental stressors (see Table 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.[1–3]

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 2). 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 anti-oxidant molecules such as superoxide dismutase (SOD), catalase, thioredoxin, and the GSH antioxidant system. Metal ions such as arsenic(III/V) or copper(II)

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Table 1 Environmental stressors that induce oxidative stress

Sources	
Metals	Antimony (Sb), arsenic (As), beryllium (Be), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), lead (Pb), mercury (Hg), nickel (Ni), vanadium (V)
Particulate matter and smoke	PM10, PM2.5, carbon monoxide (CO) sulfur dioxide (SO_2), nitrogen oxides (NOx), ozone (O_3), asbestos
Agriculture-related chemicals	Pesticides, fungicides
Persistent organic pollutants	Aldrin, chlordane, DDT, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, polychlorinated biphenyls, polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, toxaphene, carcinogenic polycyclic aromatic hydrocarbons, certain brominated flame-retardants, organometallic compounds such as tributylin TBT
Hormones and environmental hormones (endocrine disrupting chemicals)	Estradiol, dehydrotestosterone, bisphenols, phthalates
Physical agents	Burn Radiation UV radiation

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.[4–7] Non-metal 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.[8–11] 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 *trans*-regulatory factors or new biomarkers induced by exposure to environmental stressors.

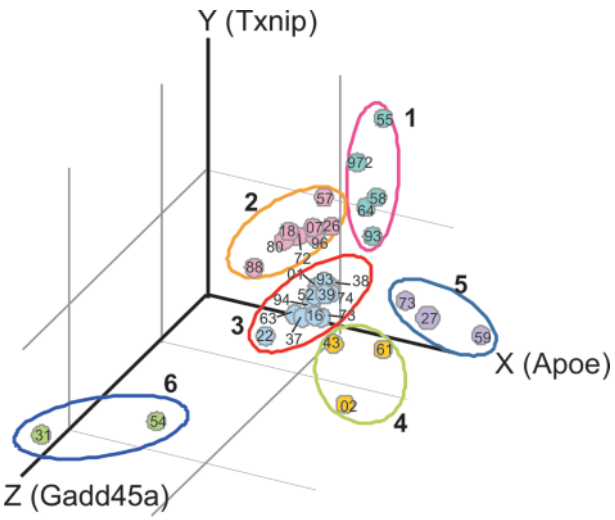

Figure 1 Principal component analysis of oxidative stress-induced genes extracted from 33 independent datasets in GEO. Numbers indicate the last two or three digits of GEOID

Microarray technology has been used in environmental toxicology and biology studies and has led to the establishment of gene expression signatures profiling the toxicity of environmental stressors.[12,13] Statistical methods used for DNA microarray studies are mostly multivariate approaches. Although basic methods treat genes as traits, which are consistent with the rules of experimental design, several approaches have been developed using expression ratio datasets. 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 (Fig. 1).[14,15] 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.[16,17] Bayesian network techniques can provide predictive information related to the relations between agents and gene expression signatures in life science fields.[18–20]

Toyokuni[21] first proposed a new science field – oxygenomics – which is defined as a research area

Table 2 Core oxidative stress pathways

Categorical pathway 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
• Anti-apoptosis genes
• Genes with antioxidant and superoxide dismutase activity
• Genes with glutathione peroxidase, oxidoreductase, 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
• Anti-apoptosis
• 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, bone morphogenetic protein (BMP) family members, growth differentiation factor (GDF), 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, NF-κB signaling pathway, TNF superfamily members, TNFr1 and TNFr2 signaling pathway, inflammatory response, transcription regulators
Mitochondria
• Mitochondrial processing, mitochondrial transportation, fatty acid biosynthesis

studying the localization of oxidative DNA damage in the genomes of living cells. Oxygenomics is becoming a significant strategy for discovery of important biomarkers and for evaluation of risks and effects.

This review addresses various environmental stressor-induced toxicities in rats and humans to elucidate the molecular mechanisms underlying toxicity-induced oxidative stress.

Categorical pathways in oxygenomics

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, aging, and some toxicant-induced apoptosis.[3,25,36] Our survey of microarray databases and many other published references has revealed the categorical pathways induced by oxidative stress, as presented in Table 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 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 co-ordinating 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 *trans*-activation 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 nitric oxide (NO), which can generate other reactive nitrogen species (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 mitochondria-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 nitric oxide 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 up-regulating 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] Reactive oxygen species (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 as a hallmark of disease progression.[52–53] In ovarian cancer, over-expression of FOXG1 contributes to TGF-β resistance through inhibition of p21WAF1/CIP1 expression, which is repressed by p53.[54] Tumor necrosis factor (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.[55] 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 are producible 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.[56–59] Mitochondrial dysfunction causes the onset of some diseases.[60–63] 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.[64]

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 up-regulation of tumor promoters and down-regulation of tumor suppressor genes; the DNA damage might, therefore, be implicated in cancer development. This review 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.

Oxidative stress responsiveness in different conditions in rats

From the Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/gds>), 33 independent microarray gene expression data with the same platform GPL341 (Affymetrix) sets in rats were downloaded for this study. All datasets were normalized across all arrays using Z-score transformation methods after combination with respect to probe IDs. The normalized values were filtered with oxidative-related genes listed in this work (see Supplement T2) and then the top 10 genes from up-regulated and down-regulated genes were chosen to analyze gene expression signatures (Table 3). The selected genes were classified 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 datasets in this work were almost all of short-period exposure using *in vivo* and *in vitro* culture systems of rats. It is noteworthy that 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, in cluster 1 (GDS964,[65] GDS972,[66] GDS1393,[67] GDS2555,[68] GDS2558,[69]), GPXs, NOS, and NOX were up-regulated, suggesting that environmental stessors in the cluster 1 can activate the NO signaling that leads to inflammation or other cellular damage. Thioredoxin interacting protein, Txnip, was identified as a unique gene in this category. In cluster 2 (GDS696,[70] GDS880,[71] GDS1518,[72] GDS1626,[73] GDS2107,[74] GDS2372,[75] GDS2457,[76] GDS2688[77]), Rad23, Rad50, 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,[78] GDS1452[79], GDS1922,[80] GDS2037,[81] GDS2073,[82] GDS2093,[83] GDS 2194,[84], GDS2616,[85] GDS2639,[86] GDS2774,[87] GDS2901,[88] GDM1038,[89]), Gadd45a, Nthl1, Mgmt, Mpp4, Chek1 Cry2, Txnrd1 were observed as up-regulated genes. Since these genes interact with DNA repair and p53 signaling activated, it is possible that environmental stressors in the cluster 3 cause DNA damage and remodeling. In cluster 4 (GDS902,[90] GDS2243,[91] GDS2361,[92]), DNA replication proteins Pinx1 and Slk were detected as unique genes. Especially, 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. Some of the genes in cluster 5 (GDS1027,[93] GDS1273,[94] GDS1959[95]) overlapped with clusters 1 and 3. In cluster 6 (GDS1354,[96] GDS2231[97]), some genes overlapped with clusters 2 and 4. However, Vim was detected as a unique gene in GDS1354, which is an experiment in cirrhotic rats,[96] and up-regulation of this gene was also observed in renal cell carcinoma,[98] cerebral tumors,[99] germ cells, and trophoblastic neoplasms.[100]

Oxidative stress-induce gene expression signatures 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 co-ordinates 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 earlier in this review.[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. The

Table 3 The top 10 up-regulated and down-regulated genes in the clusters analyzed

Cluster	GEOID	Environmental stressors (target organ or tissues)	Up-gene	Down-gene
1	GDS964	Methylprednisolone (kidney)	Apoe, Gpx2, Ngb, Nos2, Prdx6, Tmod1, Tnp1, Tpo	Brca2, Cry2, Fen1, Hus1, Ptgs2, Pttg1, Rad50, Srxn1, Xrcc6
	GDS972	Methylprednisolone (liver)	Aass, Atrx, Ncf1, Nqo1, Scd1, Slc41a3, Srd5a2, Tmod1, Tnp1	Chek1, Cry2, Lig1, Mgmt, Pold1, Pold3, Rad50, Rad52, Smc3, Xrcc6
	GDS1393	Streptozotocin (penile cavernosal)	Apc, Cat, Duox2, Gpx2, Gpx6, Gsr, Lpo, Slc38a1, Smc3, Tpo	Atrx, Gpx7, Nos2, Park7, Ptgs2, Scd1, Slc38a4, Slc41a3, Srxn1, Zmynd17
	GDS2555	Trimethyltin (hippocampus)	Apex1, Dnm2, Fancc, Gpx7, Lpo, Mgmt, Park7, Prnp, Txnip, Ucp3	Apc, Apoe, Hbz, Mpp4, Ptgs2, Smc3, Srd5a2, Tnp1, Tpo
	GDS2558	Octreotide (gastric ECL)	Brca1, Brca2, Dnm2, Duox2, Msh2, Nox4, Tmod1, Tpo, Xirp1	Apex1, Atrx, Cry2, Gpx6, Nos2, Slc38a1, Slc38a4, Slk, Tmod1, Tpo
2	GDS696	Fibronectin (ventricular myocytes)	Apoe, Atrx, Chaf1a, Ngb, Rad51c, Smc3, Srxn1, Tpo, Zmynd17	Actb, Atrx, Gsr, Mutyh, Ngb, Prdx6, Rad52, Smc3, Tpo, Txnrd1
	GDS880	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
	GDS1518	Heregulin (ureteric buds)	Dhcr24, Hus1, Ldha, Mif, Park7, Rad1, Rad50, Scd1, Tdg, Ung	Actb, Atrx, Nos2, Nox4, Nqo1, Ptgs1, Rad23a, Srxn1, Txnrd1
	GDS1626	Kainic acid (hippocampi)	Apoe, Brca2, Ncf1, Nox4, Pold1, Rad23a, Rad50, Rad51c, Srd5a2, Tmod1	Chaf1a, Hbz, Lpo, Mb, Pold3, Tnp1, Tpo, Ucp3, Ung, Zmynd17
	GDS2107	Ethanol (pancreas)	Apoe, Atrx, Hbz, Ogg1, Ptgs2, Scd1, Srxn1, Tmod1, Txnrd2, Zmynd17	Cry2, Hus1, Mb, Msh2, Nox4, Nthl1, Prdx6, Rad52, Slk, Srd5a2
	GDS2372	Sulfur dioxide (lung)	Aass, Brca1, Cry2, Hus1, Nos2, Ptgs2, Pttg1, Rad50, Tpo, Zmynd17	Apex1, Brca2, Gpx6, Nos2, Nox4, Rad23a, Rad51c, Srd5a2, Tnp1, Tpo
	GDS2457	Hypoxia (adrenal gland)	Chaf1a, Duox2, Ldha, Ngb, Pold3, Rad23a, Slc41a3, Tpo, Txnrd2	Aass, Apc, Apoe, Atrx, Cry2, Lpo, Nox4, Rad52, Srd5a2, Tnp1
	GDS2688	Methylprednisolone (skeletal muscles)	Aass, Atrx, Hbz, Ngb, Rad1, Scd1, Slc38a5, Tmod1, Tpo, Xirp1	Als2, Atrx, Brca2, Cat, Gsr, Ncf1, Nox4, Nqo1, Slc41a3, Trpc2
3	GDS1363	Forskolin (pheochromocytoma cell)	Aass, Apex1, Brca1, Chek1, Duox2, Gpx2, Hbz, Nxn, Ptgs1, Pttg1	Atrx, Cat, Cygb, Ehd2, Gpx3, Gpx4, Gpx7, Scd1, Sod3, Vim
	GDS1452	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
	GDS1922	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
	GDS2037	Angiopoietin-1 (aortic rings)	Apex1, Dnm2, Mgmt, Ngb, Pold3, Rad50, Slc38a1, Srd5a2, Srxn1, Ucp3	Atrx, Brca2, Chaf1a, Gpx6, Mb, Nox4, Rad23a, Slk, Tpo, Zmynd17
	GDS2073	Isoflurane (basolateral amygdalae)	Brca2, Gpx2, Ift172, Mif, Nos2, Pttg1, Rad1, Rad51c, Tpo, Ung	Atrx, Atrx, Gsr, Nox4, Pold3, Prnp, Ptgs2, Scd1, Smc3, Xrcc6
	GDS2093	Fe-deficiency (jejunum)	Aass, Gadd45a, Gsr, Nqo1, Srxn1, Tdg, Tmod1, Txnrd1, Xrcc1,	Gpx7, Hba-a2, Lpo, Mgmt, Nthl1, Pms2, Rad52, Smc3, Xpc, Xrcc6
	GDS2194	Pregnenolone16alpha-carbonitrile (liver)	Dnm2, Gpx6, Lpo, Nqo1, Prdx5, Ptgs2, Scd1, Srxn1, Tpo, Txnrd1	Aass, Als2, Apoe, Hbz, Nos2, Rad51c, Slc38a5, Srd5a2, Tpo
	GDS2616	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
	GDS2639	Genistein (mammary epithelial cells)	Atrx, Brca2, Hba-a2, Ngb, Rad23a, Rad52, Smc3, Tpo, Ung, Zmynd17	Apex1, Brca1, Gpx6, Lpo, Pttg1, Slc38a4, Srd5a2, Tnp1, Tpo
	GDS2774	Aging (hippocampi)	Atrx, Ehd2, Gadd45a, Gtf2h1, Mgmt, Ncf1, Nthl1, Ptgs2, Pttg1, Srxn1	Ercc6, Mlh1, Pms2, Rad50, Rad52, Slc38a1, Trpc2, Txnip, Wrnip1, Xpc
,	GDS2901	Depolarization. (midbrain)	Apc, Apoe, Atrx, Brca1, Pold3, Ptgs2, Rad23a Slc38a4, Smc3, Zmynd17	Apex1, Atrx, Chaf1a, Gpx2, Hba-a2, Nos2, Pttg1, Srxn1, Tmod1, Tnp1
	GSM1038	Aristolochic acid (kidney)	Apoe, Atrx, Cry2, Ngb, Ppp1r15b, Scd1, Srxn1, Tpo	Apoe, Atrx, Fen1, Gadd45a, Gpx6, Ift172, Pold3, Rad52, Txnip, Zmynd17
4	GDS902	Pyridine activator (ventricular myocytes)	Aass, Chaf1a, Dhcr24, Nthl1, Pinx1, Pold3, Rad52, Scd1, Slc38a1, Xirp1	Apex1, Brca2, Cry2, Gpx6, Hus1, Lpo, Mutyh, Pold1, Rad51c, Tpo
	GDS2243	Re-innervation (tibialis anterior muscles)	Apex1, Atrx, Chek1, Gpx6, Mgmt, Ncf1, Nox4, Pold3, Smc3, Tnp1	Atrx, Brca1, Chaf1a, Lpo, Nthl1, Rad50, Slc41a3, Txnrd2, Ung, Zmynd17
	GDS2361	Hyperinsulinemia (kidney)	Apoe, Chaf1a, Gpx6, Hba-a2, Lpo, Ngb, Ptgs2, Scd1, Slk, Srd5a2	Apc, Atrx, Duox2, Hbz, Mb, Ncf1, Slc38a4, Tmod1, Tnp1, Txnip

Table 3 (cont'd) The top 10 up-regulated and down-regulated genes in the clusters analyzed

Cluster	GEOID	Environmental stressors (target organ or tissues)	Up-gene	Down-gene
5	GDS1027	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
	GDS1273	Amoxicillin (intestine)	Apc, Apoe, Atrx, Lpo, Mutyh, Slc38a4, Tnp1, Tpo	Apex1, Chaf1a, Cry2, Gpx2, Ngb, Nox4, Scd1, Tpo, Trpc2, Zmynd17
	GDS1959	Ischemia (heart)	Apc, Apoe, Gpx7, Nos2, Nox4, Nxn, Prdx4, Rad52, Scd1, Smc3	Atrx, Brca1, Chaf1a, Hus1, Lpo, Pold1, Prdx5, Rad51c, Slc38a4, Xirp1
6	GDS1354	Carbon tetrachloride (liver)	Chaf1a, Ehd2, Gpx2, Hba-a2, Ncf1, Prnp, Ptgs2, Slc38a4, Vim, Zmynd17	Apoe, Dpagt1, Gab1, Hus1, Nos2, Nxn, Ptgs1, Slk, Trpc2, Txnip
	GDS2231	Dexamethasone (marrow-derived stromal cells)	Apoe, Ehd2, Gpx6, Mgmt, Mpp4, Srd5a2, Tmod1, Tpo	Apex1, Apoe, Chaf1a, Dnm2, Nos2, Rad50, Rad51c, Slk, Smc1a, Smc3

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 (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.[101–103] The 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 pro-oxidant and antioxidant genes. A new refined model for p53 activation includes three key steps: (i) p53 stabilization; (ii) anti-repression; and (iii) promoter-specific activation. Among the three steps, most environmental stressors contribute mainly to p53 stabilization and promoter-specific activation. Several reports describe that small 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.[104–106]

Therefore, to understand how stress-induced genes are downstream within the p53 pathway, we analyzed gene expression of p53 signaling pathways in array datasets GDS2780[107] and GSE7967[108] that had been obtained from the GEO database. In the GDS2780 study, six heavy metals and three organic compounds that were exposed in liver carcinoma HepG2 cells (Fig. 2A) responded dramatically to gene expression of CHK1, CHK2, Cyclin B Cdc2 p21, p53R2, Cop1-1, and Gadd45. 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;[109–113] 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 procession to apoptosis.[114–116] Consequently, exposure of cultured human cells to heavy metals dramatically altered the gene expression of oxidative-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-κB signaling in infants born to mothers exposed to arsenic at high concentration. Therefore, after downloading the datasets, 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 (As) exposure-induced responses were visualized in the p53 signaling pathway map (Fig. 2B). 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 down-regulation of p53 facilitates apoptosis and promotes cell proliferation.

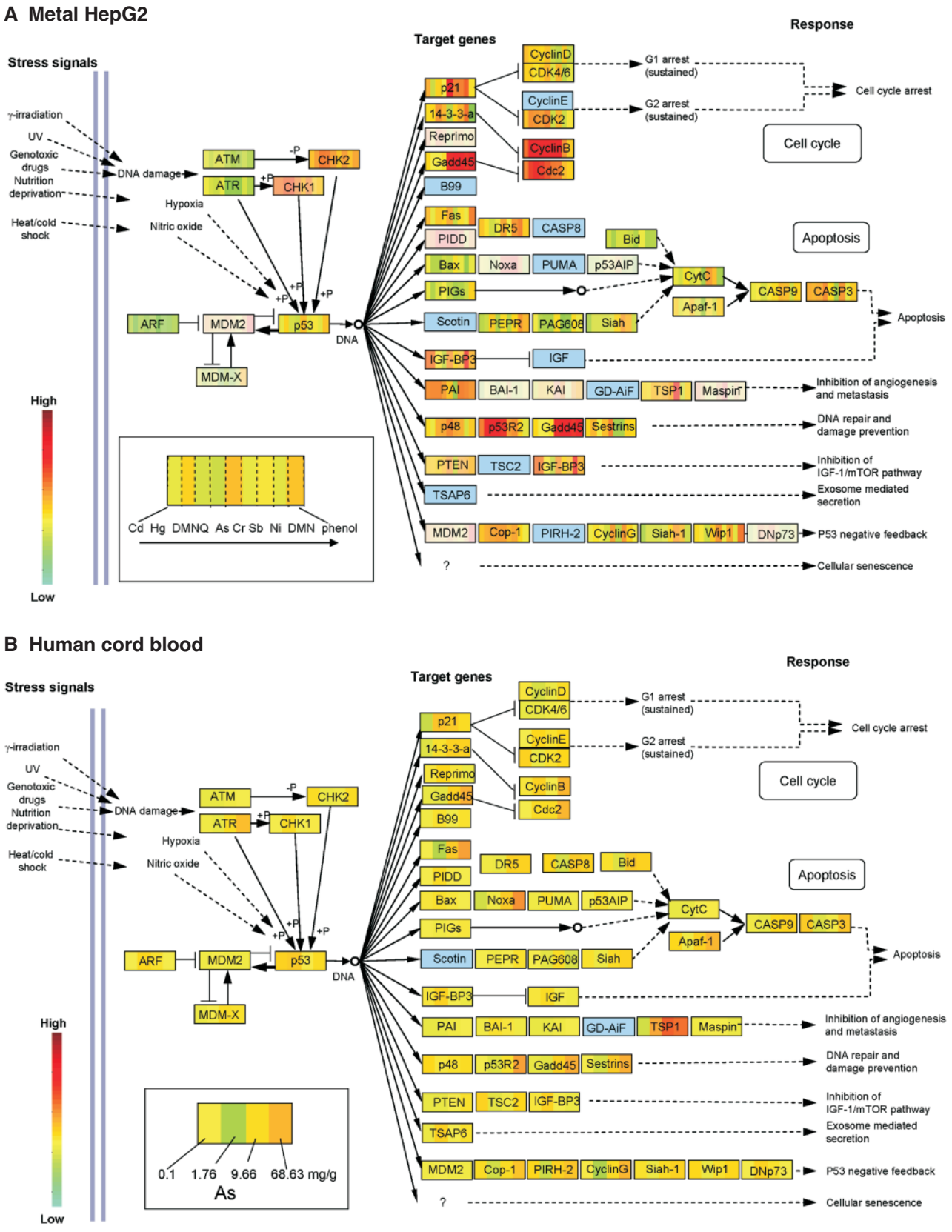

Figure 2 Oxidative gene signature in the p53 signaling pathway pathway in array datasets GDS2780[107](A) and GSE7967[108](B). (A) Heavy metals and organic compounds used in the HepG2 study. Gene expression levels in each box corresponding to each gene symbol are aligned from the left as Cd, Hg, 2,3-dimethoxy-1,4-naphthoquinone (DMNQ), As Cr, Sb, Ni, and DMN, phenol. (B) Human umbilical cord blood. Gene expression levels presented from the left are for 0.1, 1.76, 9.66, and 68.63 mg/g blood arsenic concentration

Previous works described in our study showed that GSS (glutathione synthetase) and PRDX2 (peroxiredoxin 2) regulated TNF receptor-1 associated protein (TRADD), nucleoside diphosphate linked moiety X-type motif 1 (NUDT1), SOD1, and insulin induced gene 1 (INSIG1) in the low-exposure group (mean blood concentration 0.142 μg/g), and that NUDT1 regulated TRADD, TXNRD2, 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.[17,117–119] In fact, NUDT1 is a DNA repair and recombination protein. The H_2O_2 treatment significantly increased this gene and other oxidative-stress genes involved in cell cycle arrest.[120] 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. In addition, TGF-β and TNF signaling were not strongly respondent in this re-analysis, although another paper has described pathways that are shared by oxidatively stressed and early-onset breast cancer associated interactions between TGF-β and TNF signaling.[121] Datasets used in this review are 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.

Conclusions

Herein, based on recent advances, we surveyed gene expression signatures of environmental stressor-induced oxidative stress and proposed categorical pathways and canonical pathways of oxidative stress in rodent and human systems. Analyses of gene expression signatures in environmental 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. Although we did not survey anti-oxidative stress responses induced by environmental stressors in this review, anti-oxidation systems such as the NRF2-keap1 system should be discussed for association with p53 pathways in an other review. This will take many more genetic and reverse genetic analyses, combined with functional analysis studies. Helped by complementary analyses in environmental stressor or environmental stressor-related disease, we expect soon to see the first attempts to predict influences induced by environmental stressors, taking into account the wealth of experimental data gathered. Although this might uncover interesting feed-forward and feed-back mechanisms, it will take more time to link these signaling interactions to the cell behaviors that control the different aspects of oxygenomics discussed here. It is important to realize that oxygenomics is integral to profiling effects of environmental stressors, which all need to be further classified in this way.

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SUPPLEMENTAL MATERIALS
Supplement T1 – oxidative stress pathways

Categorical pathways Canonical pathway (orthology)	Gene name
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
Anti-apoptosis	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 PCR array	
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
Anti-apoptosis 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
Mitochondrion protein import	AIP, COX10, COX18, DNAJC19, FXC1 (TIMM10B), GRPEL1, HSPD1, MIPEP, SH3GLB1
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 co-factors	CREBBP, DR1, ENO1, EP300, EPAS1, HTATIP, RARA
Transcription factors	ARNT2, BHLHB2, CREBBP, ENO1, EP300, EPAS1, HIF1A, HIF3A, KHSRP, MYBL2, PPARA, RARA
Other transcription factors & regulators	HIF1AN, NOTCH1
Anti-apoptosis	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 PCR array	
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 & 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	FASLG, LTA, TNFSF10, TNFSF14, TNFSF8,FAS, TNFRSF10A, TNFRSF10B, TNFRSF19, TNFRSF25, CD27 (TNFRSF7), TNFRSF9, TRADD,CASP3, CRADD, FADD, IKBKG, TRAF3
Other Apoptosis Genes	CD40, LTBR, NGFR, TNFRSF10C, TNFRSF11B, TNFRSF12A, TNFRSF14, TNFRSF1A, TNFRSF1B, TNFRSF21, DFFA, PAK1, TRAF2, NFKBIA, TRAF1
Caspases	CASP2, CASP3, CASP8
Caspase Activation	TNFSF15, TNFRSF10A, TNFRSF10B
Caspase Inhibition	TNFSF14, CD27 (TNFRSF7)
Anti-apoptosis Genes	CD40LG, TNF, TNFSF18, FAS, TNFRSF10D, TNFRSF18, TNFRSF6B, CD27 (TNFRSF7),BAG4, CASP2, NFKB1, TNFAIP3
NF-κB Signaling	FASLG, TNF, TNFSF10, TNFSF14, TNFSF15, CD40, EDA2R, LTBR, TNFRSF10A, TNFRSF10B, TNFRSF1A, CD27 (TNFRSF7), TRADD, CASP8, FADD, CHUK, IKBKB, IKBKG, NFKBIA, TNFAIP3

Other TNF Superfamily Members	LTB, PGLYRP1, TNFSF11, TNFSF12, TNFSF13, TNFSF13B, TNFSF4, TNFSF5IP1
Other TNF Receptor Superfamily Members	TNFRSF11A, TNFRSF13B, TNFRSF13C, TNFRSF17, TNFRSF19L, TNFRSF4, TNFRSF8
TNFR1 Signaling	ARHGDIB, CAD, HRB, LMNA, LMNB1, LMNB2, MADD, MAP3K7, PAK2, PRKDC, SPTAN1
TNFR2 Signaling	DUSP1, HRB, IKBKAP, MAP3K1, MAP3K14, TANK
JNK Signaling	EDA2R, TNFRSF19, CD27 (TNFRSF7), MAP2K4, MAPK8, PAK1
Transcription Regulators	JUN, PARP1, RB1, TNF, TNFRSF1A, IKBKB, IKBKG, NFKB1, NFKBIA

Supplement T2 – Lists of oxidative-response genes in the pathways shown in Supplement T1

Unigene	Symbol	Description
Hs.470316	ACVR1	Activin A receptor, type I
Hs.470174	ACVR2A	Activin A receptor, type IIA
Hs.591026	ACVRL1	Activin A receptor type II-like 1
Hs.112432	AMH	Anti-Mullerian hormone
Hs.659889	AMHR2	Anti-Mullerian hormone receptor, type II
Hs.533336	BAMBI	BMP and activin membrane-bound inhibitor homolog (*Xenopus laevis*)
Hs.654541	BGLAP	Bone gamma-carboxyglutamate (gla) protein
Hs.1274	BMP1	Bone morphogenetic protein 1
Hs.73853	BMP2	Bone morphogenetic protein 2
Hs.387411	BMP3	Bone morphogenetic protein 3
Hs.68879	BMP4	Bone morphogenetic protein 4
Hs.296648	BMP5	Bone morphogenetic protein 5
Hs.285671	BMP6	Bone morphogenetic protein 6
Hs.473163	BMP7	Bone morphogenetic protein 7
Hs.660998	BMPER	BMP binding endothelial regulator
Hs.524477	BMPR1A	Bone morphogenetic protein receptor, type IA
Hs.598475	BMPR1B	Bone morphogenetic protein receptor, type IB
Hs.471119	BMPR2	Bone morphogenetic protein receptor, type II (serine/threonine kinase)
Hs.437705	CDC25A	Cell division cycle 25 homolog A (*S. pombe*)
Hs.370771	CDKN1A	Cyclin-dependent kinase inhibitor 1A (p21, Cip1)
Hs.72901	CDKN2B	Cyclin-dependent kinase inhibitor 2B (p15, inhibits CDK4)
Hs.248204	CER1	Cerberus 1, cysteine knot superfamily, homolog (*Xenopus laevis*)
Hs.166186	CHRD	Chordin
Hs.172928	COL1A1	Collagen, type I, alpha 1
Hs.489142	COL1A2	Collagen, type I, alpha 2
Hs.443625	COL3A1	Collagen, type III, alpha 1
Hs.304682	CST3	Cystatin C
Hs.419	DLX2	Distal-less homeobox 2
Hs.76753	ENG	Endoglin
Hs.656395	EVI1	Ecotropic viral integration site 1
Hs.709461	FKBP1B	FK506 binding protein 1B, 12.6 kDa
Hs.25647	FOS	V-fos FBJ murine osteosarcoma viral oncogene homolog
Hs.9914	FST	Follistatin
Hs.279463	GDF2	Growth differentiation factor 2
Hs.86232	GDF3	Growth differentiation factor 3
Hs.1573	GDF5	Growth differentiation factor 5
Hs.492277	GDF6	Growth differentiation factor 6
Hs.447688	GDF7	Growth differentiation factor 7
Hs.440438	GSC	Goosecoid homeobox
Hs.632033	HIPK2	Homeodomain interacting protein kinase 2
Hs.504609	ID1	Inhibitor of DNA binding 1, dominant negative helix-loop-helix protein
Hs.180919	ID2	Inhibitor of DNA binding 2, dominant negative helix-loop-helix protein
Hs.160562	IGF1	Insulin-like growth factor 1 (somatomedin C)
Hs.450230	IGFBP3	Insulin-like growth factor binding protein 3
Hs.654458	IL6	Interleukin 6 (interferon, beta 2)
Hs.407506	INHA	Inhibin, alpha
Hs.583348	INHBA	Inhibin, beta A
Hs.1735	INHBB	Inhibin, beta B
Hs.536663	ITGB5	Integrin, beta 5
Hs.654470	ITGB7	Integrin, beta 7
Hs.714791	JUN	Jun oncogene
Hs.25292	JUNB	Jun B proto-oncogene
Hs.656214	LEFTY1	Left-right determination factor 1
Hs.713533	LTBP1	Latent transforming growth factor beta binding protein 1
Hs.512776	LTBP2	Latent transforming growth factor beta binding protein 2
Hs.466766	LTBP4	Latent transforming growth factor beta binding protein 4
Hs.202453	MYC	V-myc myelocytomatosis viral oncogene homolog (avian)
Hs.654502	NBL1	Neuroblastoma, suppression of tumorigenicity 1
Hs.370414	NODAL	Nodal homolog (mouse)
Hs.248201	NOG	Noggin
Hs.268490	NR0B1	Nuclear receptor subfamily 0, group B, member 1
Hs.1976	PDGFB	Platelet-derived growth factor beta polypeptide (simian sarcoma viral (v-sis) oncogene homolog)
Hs.77274	PLAU	Plasminogen activator, urokinase
Hs.149261	RUNX1	Runt-related transcription factor 1
Hs.414795	SERPINE1	Serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
Hs.604588	SMAD1	SMAD family member 1
Hs.12253	SMAD2	SMAD family member 2
Hs.714621	SMAD3	SMAD family member 3
Hs.75862	SMAD4	SMAD family member 4
Hs.167700	SMAD5	SMAD family member 5

Hs.189329	SMURF1	SMAD specific E3 ubiquitin protein ligase 1
Hs.643910	SOX4	SRY (sex determining region Y)-box 4
Hs.642990	STAT1	Signal transducer and activator of transcription 1, 91kDa
Hs.645227	TGFB1	Transforming growth factor, beta 1
Hs.513530	TGFB1I1	Transforming growth factor beta 1 induced transcript 1
Hs.507916	TSC22D1	TSC22 domain family, member 1
Hs.133379	TGFB2	Transforming growth factor, beta 2
Hs.592317	TGFB3	Transforming growth factor, beta 3
Hs.369397	TGFBI	Transforming growth factor, beta-induced, 68kDa
Hs.494622	TGFBR1	Transforming growth factor, beta receptor 1
Hs.604277	TGFBR2	Transforming growth factor, beta receptor II (70/80kDa)
Hs.482390	TGFBR3	Transforming growth factor, beta receptor III
Hs.446350	TGFBRAP1	Transforming growth factor, beta receptor associated protein 1
Hs.373550	TGIF1	TGFB-induced factor homeobox 1
Hs.534255	B2M	Beta-2-microglobulin
Hs.412707	HPRT1	Hypoxanthine phosphoribosyltransferase 1
Hs.523185	RPL13A	Ribosomal protein L13a
Hs.592355	GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
Hs.520640	ACTB	Actin, beta
N/A	HGDC	Human Genomic DNA Contamination
Hs.431048	ABL1	C-abl oncogene 1, receptor tyrosine kinase
Hs.601206	ANKRD17	Ankyrin repeat domain 17
Hs.73722	APEX1	APEX nuclease (multifunctional DNA repair enzyme) 1
Hs.367437	ATM	Ataxia telangiectasia mutated
Hs.271791	ATR	Ataxia telangiectasia and Rad3 related
Hs.533526	ATRX	Alpha thalassemia/mental retardation syndrome X-linked (RAD54 homolog, *S. cerevisiae*)
Hs.194143	BRCA1	Breast cancer 1, early onset
Hs.519162	BTG2	BTG family, member 2
Hs.292524	CCNH	Cyclin H
Hs.184298	CDK7	Cyclin-dependent kinase 7
Hs.24529	CHEK1	CHK1 checkpoint homolog (*S. pombe*)
Hs.291363	CHEK2	CHK2 checkpoint homolog (*S. pombe*)
Hs.135471	CIB1	Calcium and integrin binding 1 (calmyrin)
Hs.249129	CIDEA	Cell death-inducing DFFA-like effector a
Hs.151573	CRY1	Cryptochrome 1 (photolyase-like)
Hs.290758	DDB1	Damage-specific DNA binding protein 1, 127kDa
Hs.505777	DDIT3	DNA-damage-inducible transcript 3
Hs.339396	DMC1	DMC1 dosage suppressor of mck1 homolog, meiosis-specific homologous recombination (yeast)
Hs.435981	ERCC1	Excision repair cross-complementing rodent repair deficiency, complementation group 1 (includes overlapping antisense sequence)
Hs.487294	ERCC2	Excision repair cross-complementing rodent repair deficiency, complementation group 2
Hs.498248	EXO1	Exonuclease 1
Hs.591084	FANCG	Fanconi anemia, complementation group G
Hs.409065	FEN1	Flap structure-specific endonuclease 1
Hs.292493	XRCC6	X-ray repair complementing defective repair in Chinese hamster cells 6
Hs.80409	GADD45A	Growth arrest and DNA-damage-inducible, alpha
Hs.9701	GADD45G	Growth arrest and DNA-damage-inducible, gamma
Hs.661218	GML	Glycosylphosphatidylinositol anchored molecule like protein
Hs.577202	GTF2H1	General transcription factor IIH, polypeptide 1, 62kDa
Hs.191356	GTF2H2	General transcription factor IIH, polypeptide 2, 44kDa
Hs.386189	GTSE1	G-2 and S-phase expressed 1
Hs.152983	HUS1	HUS1 checkpoint homolog (*S. pombe*)
Hs.503048	IGHMBP2	Immunoglobulin mu binding protein 2
Hs.17253	IP6K3	Inositol hexakisphosphate kinase 3
Hs.61188	XRCC6BP1	XRCC6 binding protein 1
Hs.1770	LIG1	Ligase I, DNA, ATP-dependent
Hs.463978	MAP2K6	Mitogen-activated protein kinase kinase 6
Hs.432642	MAPK12	Mitogen-activated protein kinase 12
Hs.35947	MBD4	Methyl-CpG binding domain protein 4
Hs.195364	MLH1	MutL homolog 1, colon cancer, non-polyposis type 2 (E. coli)
Hs.436650	MLH3	MutL homolog 3 (*E. coli*)
Hs.509523	MNAT1	Menage-a-trois homolog 1, cyclin H assembly factor (*Xenopus laevis*)
Hs.459596	MPG	N-methylpurine-DNA glycosylase
Hs.192649	MRE11A	MRE11 meiotic recombination 11 homolog A (*S. cerevisiae*)
Hs.597656	MSH2	MutS homolog 2, colon cancer, non-polyposis type 1 (E. coli)
Hs.280987	MSH3	MutS homolog 3 (*E. coli*)
Hs.271353	MUTYH	MutY homolog (*E. coli*)
Hs.391463	N4BP2	Nedd4 binding protein 2
Hs.492208	NBN	Nibrin
Hs.66196	NTHL1	Nth endonuclease III-like 1 (*E. coli*)
Hs.380271	OGG1	8-Oxoguanine DNA glycosylase
Hs.20930	PCBP4	Poly(rC) binding protein 4
Hs.147433	PCNA	Proliferating cell nuclear antigen
Hs.424932	AIFM1	Apoptosis-inducing factor, mitochondrion-associated, 1
Hs.111749	PMS1	PMS1 postmeiotic segregation increased 1 (*S. cerevisiae*)
Hs.632637	PMS2	PMS2 postmeiotic segregation increased 2 (*S. cerevisiae*)
Hs.225784	PMS2L3	Postmeiotic segregation increased 2-like 3
Hs.78016	PNKP	Polynucleotide kinase 3'-phosphatase
Hs.631593	PPP1R15A	Protein phosphatase 1, regulatory (inhibitor) subunit 15A
Hs.491682	PRKDC	Protein kinase, DNA-activated, catalytic polypeptide
Hs.531879	RAD1	RAD1 homolog (*S. pombe*)
Hs.16184	RAD17	RAD17 homolog (*S. pombe*)
Hs.375684	RAD18	RAD18 homolog (*S. cerevisiae*)
Hs.81848	RAD21	RAD21 homolog (*S. pombe*)
Hs.655835	RAD50	RAD50 homolog (*S. cerevisiae*)
Hs.631709	RAD51	RAD51 homolog (RecA homolog, *E. coli*) (*S. cerevisiae*)
Hs.172587	RAD51L1	RAD51-like 1 (*S. cerevisiae*)
Hs.655354	RAD9A	RAD9 homolog A (*S. pombe*)
Hs.546282	RBBP8	Retinoblastoma binding protein 8

Hs.443077	REV1	REV1 homolog (*S. cerevisiae*)
Hs.461925	RPA1	Replication protein A1, 70 kDa
Hs.408846	SEMA4A	Sema domain, immunoglobulin domain (Ig), transmembrane domain (TM) and short cytoplasmic domain, (semaphorin) 4A
Hs.591336	SESN1	Sestrin 1
Hs.211602	SMC1A	Structural maintenance of chromosomes 1A
Hs.81424	SUMO1	SMT3 suppressor of mif two 3 homolog 1 (*S. cerevisiae*)
Hs.654481	TP53	Tumor protein p53
Hs.697294	TP73	Tumor protein p73
Hs.707026	TREX1	Three prime repair exonuclease 1
Hs.191334	UNG	Uracil-DNA glycosylase
Hs.654364	XPA	*Xeroderma pigmentosum*, complementation group A
Hs.475538	XPC	*Xeroderma pigmentosum*, complementation group C
Hs.98493	XRCC1	X-ray repair complementing defective repair in Chinese hamster cells 1
Hs.647093	XRCC2	X-ray repair complementing defective repair in Chinese hamster cells 2
Hs.592325	XRCC3	X-ray repair complementing defective repair in Chinese hamster cells 3
Hs.444451	ZAK	Sterile alpha motif and leucine zipper containing kinase AZK