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 (incuding 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 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 Anti-apoptosis Induction of Apoptosis and caspase Activity Protein Biosynthesis, Phosphorylation and metabolism Cytoskeleton and Other Extracellular Molecules Cell Cycle, Cell Proliferation & Growth Factors Carbohydrate, lipid, One-carbon compound metabolism Metabolism RNA Metabolism Cardiac Excitation-Contraction (E-C) Coupling
TGFß-BMP signaling	tgf-ß superfamily, bmp (bone morphogenetic protein) family members, gdf (growth differentiation factor) , activin, and activin receptors smad family members, TGF-ß/ activin-responsive genes, bmp-responsive genes, molecules regulating signaling of the TGF -ß 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

Table 13.2 Oxidative-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
	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	IGF1R, IL6

Proliferation	
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 oxidoreductaseactivity:	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	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 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
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
TGFß BMP Signaling	
TGF-ß	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-ß/ 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	BGLAP (osteocalcin), DLX2, ID1, ID2, JUNB, SOX4, STAT1
Molecules Regulating Signaling of the TGF-ß 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	FASLG, LTA, TNFSF10, TNFSF14, TNFSF8
Caspase Activation	TNFSF15
Caspase Inhibition	TNFSF14
Anti-apoptosis Genes	CD40LG, TNF, TNFSF18
Other Apoptosis-Related Genes	CD70 (TNFSF7), TNFSF9
Inflammatory Response	CD40LG, TNF
NFκB Signaling Pathway	FASLG, TNF, TNFSF10, TNFSF14, TNFSF15
Other TNF Superfamily Members	LTB, PGLYRP1, TNFSF11, TNFSF12, TNFSF13, TNFSF13B, TNFSF4, TNFSF5IP1
Induction of Apoptosis	FAS, TNFRSF10A, TNFRSF10A, TNFRSF10B, TNFRSF19, TNFRSF25, CD27 (TNFRSF7), TNFRSF9, TRADD
Caspase Activation	TNFRSF10A, TNFRSF10B
Caspase Inhibition	CD27 (TNFRSF7)
Anti-apoptosis Genes	FAS, TNFRSF10D, TNFRSF18, TNFRSF6B, CD27 (TNFRSF7)
Other Apoptosis Genes	CD40, LTBR, NGFR, TNFRSF10C, TNFRSF11B, TNFRSF12A, TNFRSF14, TNFRSF1A, TNFRSF1B, TNFRSF21
Inflammatory Response	CD40, TNFRSF1A
NFκB Signaling Pathway	CD40, EDA2R, LTBR, TNFRSF10A, TNFRSF10B, TNFRSF1A, CD27 (TNFRSF7), TRADD
JNK Signaling Pathway	EDA2R, TNFRSF19, CD27 (TNFRSF7)
Other TNF Receptor Superfamily Members	TNFRSF11A, TNFRSF13B, TNFRSF13C, TNFRSF17, TNFRSF19L, TNFRSF4, TNFRSF8
Induction of Apoptosis	CASP3, CRADD, FADD, TRADD
Caspases	CASP2, CASP3, CASP8
Anti-apoptosis Genes	BAG4, CASP2, TNF
Other Apoptosis Genes	DFFA, PAK1, TNFRSF1A, TRAF2
Inflammatory Response	TNF, TNFRSF1A
NFκB Signaling Pathway	CASP8, FADD, TNF, TNFRSF1A, TRADD
JNK Signaling Pathway	MAP2K4, MAPK8, PAK1
Transcription Regulators	JUN, PARP1, RB1, TNF, TNFRSF1A

TNFR1 Signaling Pathway	ARHGDIB, CAD, HRB, LMNA, LMNB1, LMNB2, MADD, MAP3K1, MAP3K7, PAK2, PRKDC, SPTAN1
Induction of Apoptosis	IKBKG, LTA, TRAF3
Anti-apoptosis Genes	NFKB1, TNFAIP3
Other Apoptosis Genes	NFKBIA, TNFRSF1B, TRAF1, TRAF2
Inflammatory Response	NFKB1
NFκB Signaling Pathway	CHUK, IKBKB, IKBKG, NFKBIA, TNFAIP3
Transcription Regulators	IKBKB, IKBKG, NFKB1, NFKBIA
TNFR2 Signaling Pathway	DUSP1, HRB, IKBKAP, MAP3K1, MAP3K14, TANK

Table 13.3 Environmental stressors induce different gene expression signatures

Environmental stressors (target organ or tissues)	Up-gene	Down-gene	GEOID
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
Cluster4			
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

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

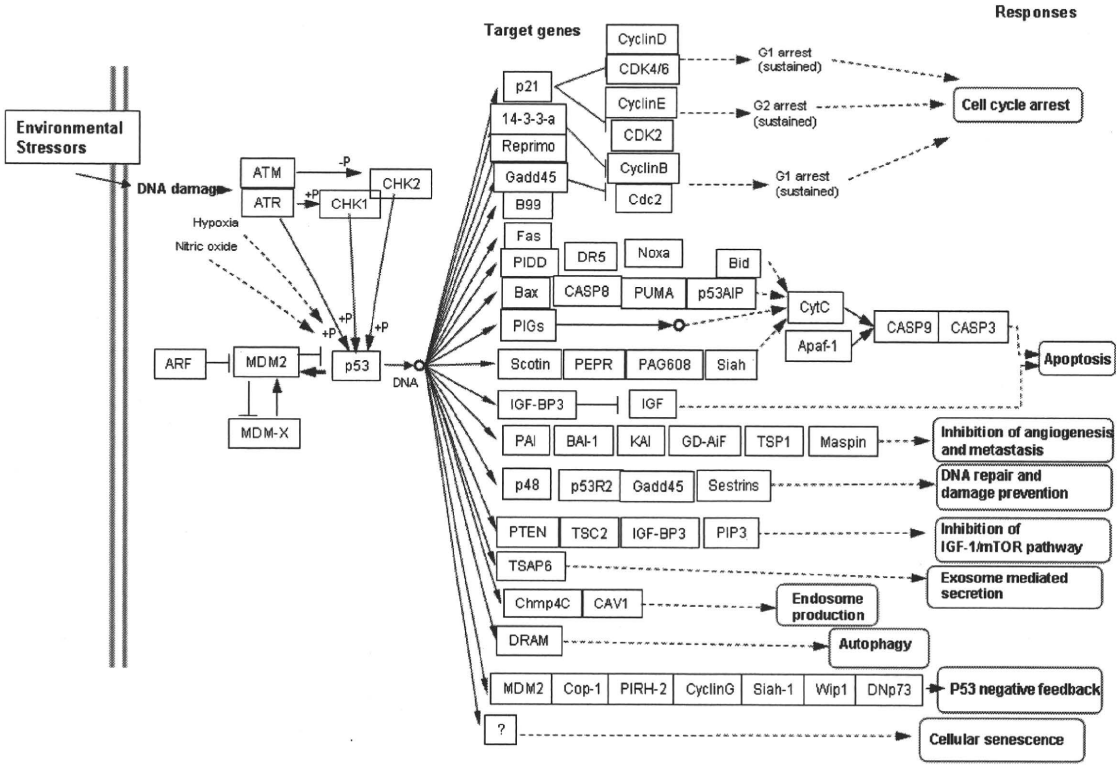

Figure 13.1. Environmental Stressors-mediated p53 signaling pathways Maps in the p53 signaling pathway partly consulted the KEGG pathway.

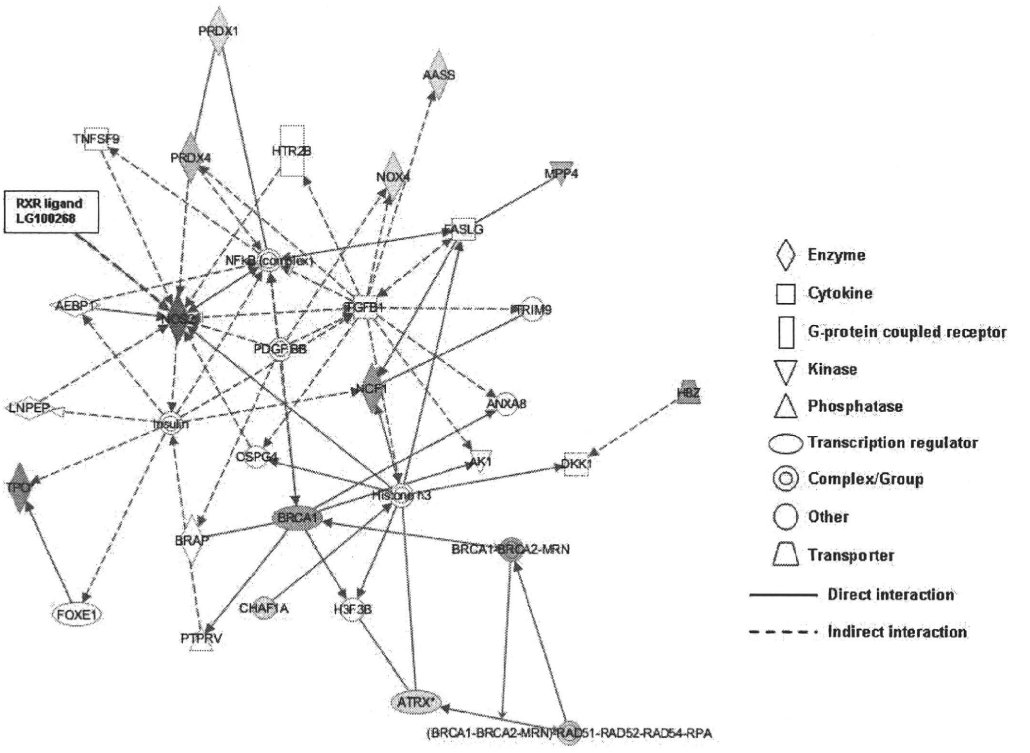

Figure 13.2. The network generated by retinoic X receptor ligand LG100268 in cluster 3 shown in Table 3 and 4: the top-rated network as an example.

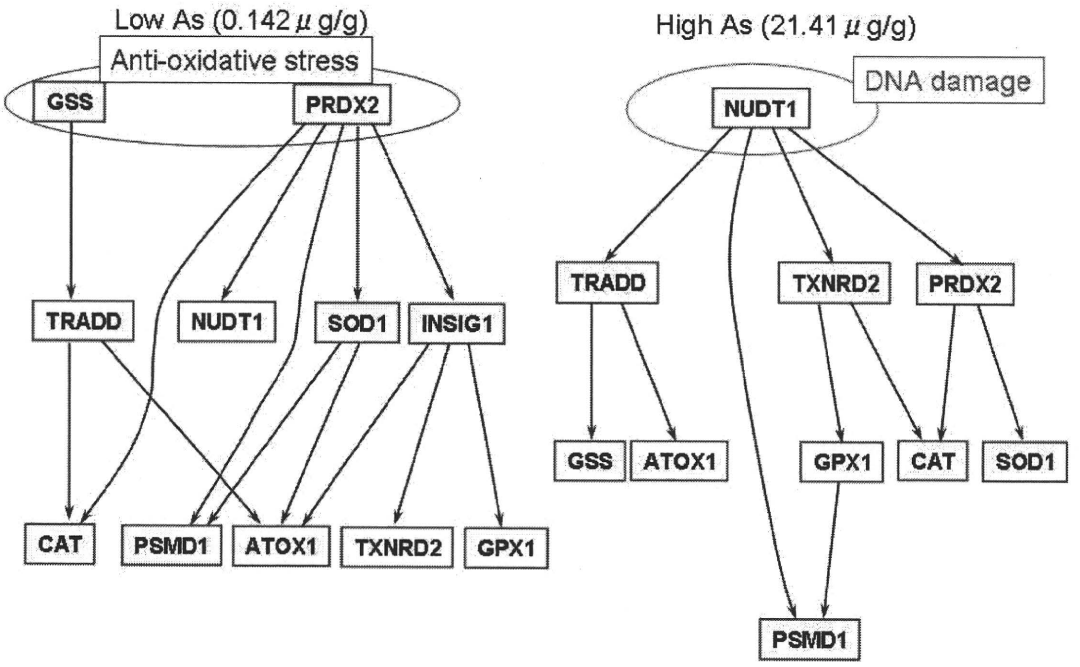

Figure 13.3. Networks for oxidative stress-related 11 genes selected from the GSE7967 study. TAO-Gen algorisms can predict different mechanisms in the low and high exposure to arsenic.

Review

Title: **Perinatal Exposure to Environmental Chemicals Induces Epigenomic Changes in Offspring**

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Running head: Environmental Factors Induced Epigenomic Changes

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Abstract

Many researchers propose that invisible internal alterations that occur through exposure to environmental factors during fetal or neonatal stages affect the risk of cancer, hypertension, and diabetes after maturation. Barker's hypothesis, which states that reduced fetal growth is strongly associated with metabolic syndromes including cardiovascular disease and diabetes, has now been widely accepted and expanded into the Developmental Origins of Health and Disease (DOHaD). Potential molecular mechanisms underlying this phenomenon include the alteration and persistence of epigenomic programming. Clear biochemical evidence has not yet been obtained in human studies; however, in laboratory animals, the fetal environment including physical and chemical factors altered epigenomic states such as DNA methylation and histone modification, and persistent changes affected specific gene expression regulation, resulting in disease susceptibility. Furthermore, in recent studies, environmental chemical exposure during pregnancy altered sperm DNA methylation patterns of male offspring, and the altered status and resulting phenotype were inherited in the next generation. Challenging and eccentric studies focusing on epigenetic transgenerational effects are currently being conducted to demonstrate the existence of Lamarckian inheritance.

Key words: epigenetics, environmental chemical, DOHaD, acquired characteristics

1. Introduction

Recently, several researchers have proposed that invisible alterations that occur as a result of exposure to environmental factors during fetal or immature stages influence the risk of cancer, hypertension, and diabetes in adulthood. The molecular bases for disease susceptibilities are linked to epigenetics [1-5]. Various environmental factors surrounding pregnant mothers and infants could be potential stimuli for epigenome alternation. For example, nutrient changes, exposure to environmental chemicals, and physical stresses such as insufficient care can alter the epigenome; thus, concern regarding chemicals that affect the health of the next generation has recently increased [6-9]. In this review, several studies conducted on animals are presented to summarize the evidence that the fetal and neonatal environment can influence epigenomic modifications retained in later stages of life.

2. Epigenome features and mechanism of their structural inheritance

In the field of epigenetic research, a considerable amount of attention has been paid to DNA methylation and histone modification because these processes are the most important epigenomic features. Variations in the amino acid acetylation/methylation status of histone octamers in the nucleosome of specific regions of chromatin and changes in DNA cytosine methylation patterns can be inherited by daughter cells after replication and cell division. Therefore, chromatin modification patterns are considered genetic information (epigenetic information or memory) [10].

DNA methylation typically occurs at CpG sites, which are 5'-CG-3' dinucleotide sequences. Cytosine nucleotides of CpG are not methylated in newly synthesized DNA strands; thus, this double stranded state is called hemi-methylated DNA. Immediately after DNA replication, hemi-methylated DNA is recognized by Np95 (UHRF1) [11], which is a component of the replication factory complex. This complex contains proliferating cell nuclear antigen (PCNA) and DNA methyltransferase 1 (DNMT1), which transfers a methyl group to the 5 position of unmethylated cytosine in the newly synthesized opposite DNA strand [12, 13]. By this simple mechanism, information with methyl-CpG patterns (methylation status) is maintained in the nucleus of the daughter cell. CpG sites occur at a relatively low frequency in the genome. Because methyl-CpG sites are more susceptible to mutation and are lost over time, CpG clusters or CpG islands are located on a specific region of the genome, *i.e.*, gene promoter and exon 1. CpG methylation negatively regulates gene expression because transcription factors cannot bind methyl-CpG containing elements [14]. Thereby, CpG islands of cell type specific regulatory genes are methylated followed by further chromatin modification. This chromatin remodeling including CpG methylation is highly correlated with cellular transformation and differentiation [15, 16].

A histone octamer composed of one H2A, one H2B, two H3, and two H4 proteins is one unit of the nucleosome. Acetylation and methylation of acidic amino acid residues (lysine and arginine) in the amino-terminals of histone H3 and H4 are major epigenetic marks. In addition, phosphorylation, ubiquitination, sumoylation, ADP-ribosylation, and biotinylation are other chemical modifications of the histone tail [17]. Histone acetylation and methylation are attuned to transcription activation and chromatin inactivation, respectively. Patterns of the covalent modifications of histone tails are extremely varied due to the number of modifiable amino acids and the possible combinations of substitution. Code information other than nucleotide sequences was advocated as the histone-code hypothesis; however, this theory is currently disregarded [18]. Nevertheless, these histone-based modification patterns must influence region specific chromatin structure, resulting in differences in the level of gene expression. Many mechanistic models have been constructed to explain how histone modification patterns are inherited by daughter cells. In particular, a random distribution model is shown in Figure 1 [19]. On the newly synthesized DNA, parental histone H3-H4 tetramers are reused for the formation of the nucleosome of daughter chromatin. At the same time, newly synthesized histone H3-H4 tetramers are incorporated into the daughter nucleosome at random. Newly synthesized histone marks, *e.g.*, acetylated lysine, are erased by histone deacetylases (HDACs). Methylated marks derived from parental histones that are next to newly incorporated histones are recognized by heterochromatin protein 1 (HP1), which forms a complex with histone methyltransferase (HMT). Subsequently, erased marks in the newly incorporated histones are quickly methylated by HMT. In this manner, histone modification patterns in a specific region of the genome are precisely copied. In addition, a semi-conservative model in which only dimers of H3-H4

tetramer are reused during nucleosome formation and an asymmetric model in which interactions between two newly synthesized DNA strands have been proposed for the regulation of histone modification copying [10, 18, 19].

Other than DNA methylation and histone modification, non-coding RNA, microRNA, and polycomb group (PcG) protein complex are epigenetic factors that modulate chromatin higher structure and influence the cellular phenotype [20, 21]. These varieties of macromolecular architectures may regulate and integrate epigenome inheritance.

3. Human disease and epigenetics

In the retrospective cohort studies of David Barker and colleagues conducted in the United Kingdom, in which the country was divided into 212 local authority areas, a strong geographical relationship was observed between ischaemic heart disease mortality rates in 1968-78 and infant mortality in 1921-25 [1]. Based on detailed epidemiological studies regarding the relationship between mother's birth weight and blood pressure, they concluded that babies who were small at birth or during infancy displayed increased rates of cardiovascular disease in adulthood [2]. Moreover, they proposed the thrifty phenotype hypothesis, in which epidemiological associations between poor fetal and infant growth and the subsequent development of type 2 diabetes and metabolic syndrome are due to the effects of poor nutrition in early life, which produces permanent changes in glucose-insulin metabolism [22]. The aforementioned theory is Barker's hypothesis, in which low maternal nutrition causes metabolic syndrome in offspring. Later termed the Developmental Origins of Health and Disease (DOHaD), the theory was expanded from Barker's hypothesis into the more general theory that health and disease susceptibility in the next generation depends on the environment surrounding the fertilized egg and fetuses in uterus, and throughout the neonatal life time [3, 4, 23].

Several attempts have recently been made to explain this phenomenon. The most reliable molecular mechanism underlying DOHaD is that epigenetic alternations that occur during the sensitive stage of fetus and newborn development persist into adulthood [5]. In human studies, clear biochemical evidence has not yet been obtained; however, in laboratory animal experiments, the fetal environment, including physical and chemical factors altered epigenomic states such as DNA methylation and histone modification and persistent changes, influenced specific gene expression regulation [5]. DOHaD has become more than a hypothesis and has been accepted by the clinical field. In the next section, animal studies related to DOHaD will be described.

Many carcinogeneses are known to be caused by aberrant DNA methylation, which is partially acquired in later stages of development. The hypermethylation of tumor suppressor genes such as *pRB* or *p16* is involved in retinoblastoma and epigenetic carcinogenesis [24, 25]. Clinical researchers are now considering epigenetic aberrations during carcinogenesis because DNA methyltransferase inhibitors are useful for cancer therapy [26, 27, 28]. Thus, concern about chemically-induced epigenetic carcinogenesis in the next generation is increasing [6, 8].

4. Animal models representing epigenomic changes due to perinatal environmental factors

Recently, a number of reports based on experimental animal models such as rats and mice demonstrated that *in utero* or neonatal nursing environmental stimuli altered DNA methylation and histone modification, which can persist into adulthood and potentially influence the phenotype [5, 29]. In most of these reports, clear and significant changes in the epigenome of specific target genes were detected. As described briefly below, in many of these reports, environmental chemicals including endocrine disruptors, which do not have mutagenic activities, were evaluated. Several leading papers are summarized in Table 1.

In rats, naturally occurring variations in maternal care influence the sensitivity of offspring to stress in adulthood. Meaney and colleagues at McGill University reported that the offspring of mothers that exhibited more licking and grooming of pups showed reduced plasma adrenocorticotropic hormone and corticosterone responses to acute stress, increased hippocampal glucocorticoid receptor (*Gr*) mRNA expression, and enhanced glucocorticoid feedback sensitivity [30]. Increased pup licking and grooming and arched-back nursing by rat mothers altered the offspring epigenome at a *Gr* gene promoter in the

hippocampus, i.e., hypomethyaltion of CpG and increased histone acetylation and transcription factor (NGFI-A) binding [31]. These studies revealed that maternal care determines offspring stress resistance in adulthood due to an altered level of epigenomic states in the brain.

Viable yellow (A^{vy}) mice are larger, obese, hyperinsulinemic, more susceptible to cancer, and shorter lived than their non-yellow siblings [32]. They are epigenetic mosaics ranging from a yellow phenotype with maximum ectopic *Agouti* gene overexpression through a continuum of mottled *Agouti/yellow* phenotypes with partial *Agouti* overexpression to a pseudoagouti phenotype with minimal ectopic expression [32, 33]. This marked phenotypic change is significantly associated with the methylation level of CpG sites in an intracisternal A particle (IAP) retrotransposon upstream of the transcription start site of the *Agouti* gene. Feeding pregnant dams methyl-supplemented diets alters the epigenetic regulation of *Agouti* expression in their offspring, as indicated by increased *Agouti/black* mottling in the direction of the pseudoagouti phenotype [34]. Maternal dietary genistein supplementation shifted the coat color of heterozygous viable yellow offspring toward pseudoagouti by hypermethylation of CpG sites in the retrotransposon of the *Agouti* gene [35]. Furthermore, maternal exposure to Bisphenol-A, an endocrine disruptor, shifted the coat color toward yellow by hypomethylation of retrotransposon CpG sites. Moreover, maternal dietary supplementation with methyl donors such as folic acid or genistein negated the DNA hypomethylating effect of Bisphenol-A [36].

Diethylstilbestrol (DES), a nonsteroidal estrogen, induces developmental anomalies in the female reproductive tract including vaginal cancer in humans perinatally exposed to DES. The expression of Homeobox gene Hoxa10, which controls uterine organogenesis, is repressed in DES-exposed mouse offspring [37]. In the mouse model, CpG methylation frequency in the Hoxa10 intron was greater in DES-exposed offspring than control mice and was accompanied by increased expression of Dnmt 1 and Dnmt 3b.

Perinatal exposure to methylmercury causes persistent changes in learning and motivational behavior in C57BL/6 mice. Behavioral alterations are associated with a decrease in brain-derived neurotrophic factor (BDNF) mRNA in the hippocampal dentate gyrus. In this mouse experiment, methylmercury exposure caused the chromatin structure at the *Bdnf* promoter region to enter into a long-lasting repressive state. In particular, DNA hypermethylation, an increase in histone H3-K27 tri-methylation, and a decrease in H3 acetylation were observed at promoter IV [38].

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic environmental pollutant. Short term exposure of preimplantation ICR mouse embryos to TCDD (10 nM) during the 1-cell stage to the blastocyst stage resulted in reduced fetal weight compared to unexposed preimplanted embryos. A decrease in the level of expression of imprinted genes H19 and Igf2 was observed, along with in an increase in the methylation frequency of the *H19/Igf2* imprint control region due to an increase in the paternal methylation status [39].

Perinatal exposure to a low dose of TCDD (environmental contamination levels) induces various types of developmental abnormalities in the offspring of laboratory animals [40, 41]. The cellular target molecule is an arylhydrocarbon receptor (Ahr), which mediates every outcome of low dose TCDD toxicity [42]. Ahr can bind to polycyclic aromatic hydrocarbon mutagens such as benz[a]pyrene (BaP) and dimethylbenzanthracene (DMBA) and can transactivate downstream target genes, including cytochrome P450 (CYP) family 1A1 and 1B1, which are phase I drug metabolizing enzymes [43]. Using its monooxygenase activity, CYPs convert the mutagen into a reactive epoxide, which produces a covalent bond to the guanine residue in DNA and results in nucleotide mutation [44]. In contrast, TCDD is a stable compound in the environment and living bodies; thus, TCDD cannot bind to DNA directly and induce mutations in nucleotide sequences. Female Sprague-Dawley rats exposed to TCDD in the fetal stage were more prone to DMBA-induced mammary tumors in adulthood than control rats [45]. Very low doses of 3,3',4,4',5-pentachlorobiphenyl (PCB126), which has a similar bioactivity to that of TCDD, had the same effect on DMBA-induced mammary tumor models when administered during pregnancy [46]. Moreover, the induction state of *Cyp1a1* and *Cyp1b1* genes in the liver were enhanced after DMBA injection [47]. The acceleration of *Cyp1* transcription resulted in higher carcinogenic susceptibility due to epigenetic alternations. In our laboratory, we demonstrated that C57BL/6J mice administered TCDD during the fetal stage showed a higher incidence of forestomach cancer due to BaP injection and enhanced induction of Cyp1a1 mRNA by TCDD re-administration in adulthood. Methyl-CpGs level of the *Cyp1a1* promoter region, which includes Ahr-binding xenobiotic response elements, is involved in TCDD induced

transcription activity in LNCaP cells [48]. The methylation frequency of the *Cyp1a1* promoter region in the liver DNA of mice exposed to TCDD was significantly lower than that of the control mice. Moreover, acetylation levels of histone H3 and H4 were significantly higher in TCDD-exposed mice (unpublished data).

In laboratory studies conducted on animals, substantial evidence suggests that perinatal exposure to chemicals alters the epigenetic program of sensitive organs and persists into adulthood, affecting disease susceptibility. The mechanism of epigenomic status modulation, CpG hyper/hypomethylation, and maintenance of increased histone acetylation/methylation is still unclear; however, investigations are currently being conducted by many researchers.

5. Transgenerational effects and possible Lamarckian inheritance

According to Barker's hypothesis, offspring phenotypes such as cardiovascular disease, risk of cancer, hypertension, and diabetes are thought to be inherited by the next generation [49]. The epigenome alternations summarized in the present review can be inherited by the later generation via germ cell lines. Skinner and colleagues at Washington State University administered endocrine disruptors, fungicide vinclozolin and pesticide methoxychlor, to pregnant Sprague-Dawley rats to investigate spermatogenic defects and other toxic endpoints in male offspring [50]. Transient exposure of vinclozolin, which does not display mutagenic activity, induced decreased spermatogenic capacity in adult phenotypes of the F_1 generation (sperm count and viability) and increased incidence of male infertility. These effects were transferred through the male germ line to nearly all of the males of subsequent generations, *i.e.*, F_1 to F_4 [50, 51]. Moreover, male rats whose progenitors had been treated with vinclozolin showed lowered mate preference from control females [52]. Using an arbitrary primer PCR and methylation sensitive restriction enzyme *Hpa* II, the authors demonstrated that the methylation pattern of lysophospholipase (LPLase) and cytokine-inducible SH2 protein genes was altered in the epididymal sperm DNA of F_2 and F_3 animals [50]. Most recently, using a methylated DNA immunoprecipitation technique based on methyl-cytosine antibodies and a promoter tilling microarray (MeDIP-Chip) procedure, Skinner and coworkers identified 52 different regions in the sperm promoter epigenome with altered methylation patterns due to maternal exposure of vinclozolin [53]. The series of studies conducted by Skinner's group suggest that a phenotype can be acquired by maternal exposure to environmental factors, leading to evolutional changes in animals. However, these studies are somewhat controversial because experiments focused on *Lplase* gene hypomethylation have not been reproduced using an identical protocol [54].

Compared to the offspring of males fed a control diet, the offspring of males fed a low-protein diet exhibited elevated hepatic expression of many genes involved in lipid and cholesterol biosynthesis and decreased levels of cholesterol esters [55]. Epigenomic profiling of offspring livers via deep sequencing technology revealed numerous changes in CpG methylation due to alterations in the paternal diet, including reproducible changes in methylation over a likely enhancer for *Ppara*, a key lipid regulator. The results indicated that the parental diet can affect cholesterol and lipid metabolism in offspring, and a model system to study the environmental reprogramming of the heritable epigenome was defined. Acquired characteristics in the next generation may be inherited through the epigenome of paternal germlines. However, differences in sperm DNA methylation were not detected via deep sequencing [55]. The epigenetic inheritance of RNA molecules has been described, and the expression of unusual *Kit* RNAs in the male germline (spermatozoon) of mice resulted in a phenotypic effect (on coat color) on the progeny of affected mice. In particular, two genetically identical mice differed phenotypically based on their parents' genotypes [56]. These reports imply that untranslated RNAs can be potent epigenetic modulators for transgenerational effects on acquired characteristics rather than DNA methylation [57].

In the middle of the last century, Lysenko, a Russian plant biologist, emphasized his theory by conducting nutritional hybrid experiments and suggested that a white albino tomato could be transformed into a red tomato. Moreover, he suggested that autumn wheat could become spring wheat by applying low temperature treatments; however, little evidence was presented in these experiments. The aforementioned work was based on Lemarckian inheritance, *i.e.*, the inheritance of an acquired phenotype. The theories of Lamarck and Lysenko have been neglected in the twentieth century. However, the name of Lysenko, a very infamous one, is rising again in this century, due to the growth of epigenetics [58]. Epigenetic transgenerational effects may alter germlines, and unknown factors other than DNA methylation or