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Environmental Genomics Related to Environmental Health Biomarker

Hyun-Mi Kim[1], Dae-Seon Kim[1] & Younghee Chung[1]

[1]Environmental Health and Safety Department, National Institute of Environmental Research, Incheon 404-708, Korea
Correspondence and requests for materials should be addressed to H.-M. Kim (hmikim@me.co.kr)

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Abstract

Biomarkers identify various stages and interactions on the pathway from exposure to disease. The three categories of biomarkers are those measuring susceptibility, exposure and effect. Susceptibility biomarkers are identifiable genetic variations affecting absorption, metabolism or response to environmental agents. Biomarkers of exposure indicate the amount of a foreign compound that is absorbed into the body. Biological measurements performed on human tissues are vastly expanding the capabilities of classical epidemiology, which has relied primarily on estimates of human exposure derived form chemical levels in the air, water, and other exposure routes. Biomarkers of exposure indicate the amount of a foreign compound that is absorbed into the body. Biological measurements performed on human tissues are vastly expanding the capabilities of classical epidemiology, which has relied primarily on estimates of human exposure derived form chemical levels in the air, water, and other exposure routes. The biomarker response is typical of chemical pollution by specific classes of compound, such as (i) heavy metals (mercury, cadmium, lead, zinc), responsible for the induction of metallothionein synthesis, and (ii) organochlorinated pollutants (PCBs, dioxins, DDT congeners) and polycyclic aromatic hydrocarbons (PAHs), which induce the mixed function oxygenase (MFO) involved in their bio transformations and elimination. Currently genomic researches are developed in human cDNA clone subarrays oriented toward the expression of genes involved in responses to xenobiotic metabolizing enzymes, cell cycle components, oncogenes, tumor suppressor genes, DNA repair genes, estrogen-responsive genes, oxidative stress genes, and genes known to be involved in apoptotic cell death. Several research laboratories in Korea for kicking off these Environmental Genomics were summarized.

Keywords: Environmental Health Biomarker, Epidemiology, Environmental Genomics, DNA Microarrays

Incorporation of the biotechnological tools of biochemistry and molecular biology into the epidemiology and environmental toxicology has provided new subcellular insights. Relying on biochemical and molecular biomarkers, health effects have been elicited by toxic exposure. Several approaches have been performed to identify and study the intermediate events between exposure state and biological effects. The new technology including DNA microarrays made a new emerging field of Environmental genomics in environmental research.

Traditional biomarkers include lead, mercury, arsenic or pesticide in blood and urine, indicating exposure to these substances in epidemiology. There are diversity for definition of biomarkers according to the fields of study. Generally, biomarkers include biochemical, physiological, morphological, and histopathological responses of organisms that signify exposure to contaminants. In biology and medicine, a biomarker can be a substance whose setection indicates a particular disease state (for example the presence of an antibody may indicate an infection). A biomarker can also be used to indicate exposure to various environmental substances in epidemiolgy and toxicology. In these cases the biomarker may be the external substance itself (e.g. asbestos particle) or a variant of the external substance processed by the body (a metabolite). In genetics, a biomarker (identified as genetic marker) is a fragment of DNA sequence that is associated with, changes susceptibility to disease or causes disease.

Categories of Environmental Health Biomarkers

Biomarkers identify various stages and interactions on the pathway from exposure to disease. The three categories of biomarkers are those measuring susceptibility, exposure and effect. Susceptibility biomarkers are identifiable genetic variations affecting absorption, metabolism or response to environmental agents. These genetic variations as polymorphism do not act

alone to trigger disease, but confer differential sensitivity to the effects of chemicals. Such environmental susceptibility genes can be contrasted to highly penetrant disease genes, such as those for Huntington's disease, cystic fibrosis and sickle cell anemia, in which a single mutation may be a predictor of disease even in the absence of an environmental exposure. Susceptibility genes are neither necessary nor sufficient to cause disease. Such genetic variations may increase the rate at which carcinogens or other harmful substances are activated, reduce an individual's ability to detoxify harmful compounds, or disable DNA repair mechanisms, tumor suppressor genes or other protective functions. One's genetic complement may affect the toxicity or potency of chemicals. Studies involving occupational exposures have found a genetic marker of susceptibility in workers with beryllosis, a potentially fatal respiratory disease caused by exposure to beryllium.

Biological measurements performed on human tissues are vastly expanding the capabilities of classical epidemiology, which has relied primarily on estimates of human exposure derived form chemical levels in the air, water, and other exposure routes. These methods require modeling or monitoring of the ambient environment and significant guesswork as to actual human exposure levels. These measurements have severe limitations, as individuals vary in their rates of absorption, metabolism and excretion of toxic substances, individuals subject to the same ambient exposure may retain different amounts of toxins in their bodies. Exposure biomarkers detected in the human body may include the parent chemical, metabolic derivatives, or early interactive products of the chemical or drug and the biological system.

Biomarkers of effect reflect changes in cells or tissues triggered by chemical exposure or changes that are qualitatively or quantitatively predictive of health impairment or potential impairment due to toxic exposure. Biomarkers of effect may measure biochemical or cellular changes, structural or functional changes in affected cells or tissues, or changes formally recognized as health impairments or clinical disease.

The most important contribution of the biomarker paradigm is the concept of a continuum of effects between environmental exposure and disease. One end represents a manifestation of overt disease, such as a cancerous tumor that may appear years after the in initial exposure. Validation is essentially a quality control process whereby each biomarker is evaluated for its reliability as a measure of exposure, effect, or susceptibility.

Several Kinds of Environmental Health Biomarkers

The biomarker response is typical of chemical pollution by specific classes of compound, such as (i) heavy metals (mercury, cadmium, lead, zinc), responsible for the induction of metallothionein synthesis, and (ii) organochlorinated pollutants (PCBs, dioxins, DDT congeners) and polycyclic aromatic hydrocarbons (PAHs), which induce the mixed function oxygenase (MFO) involved in their biotransformations and elimination. The enzyme aminolevulinic acid dehydratase (ALAD) is specifically inhibited by lead. Monoamide oxidase regulates biogenic amine concentration in the brain and peripheral tissue and has been shown to be a molecular target of mercury compounds in animal models.

Glutathione (GSH) is a low molecular weight intracellular thiol that is important in free radical scavenging, detoxification of xenobiotics, protein synthesis, DNA synthesis and repair, and cell proliferation. One consequence of exposure to a wide variety of xenobiotics, is the induction of glutathione biosynthetic enzymes, especially gamma-glutamylcysteine synthetase (GCS), the rate limiting enzyme in GSH biosynthesis. GCS is composed of heavy and light subunits, the former having all the catalytic activity of the enzyme, and the latter possessing features important in regulating certain aspects of GCS activity.

As catalogical biomarkers, Lysosomal enzymes such as hydrolases are capable of degrading biological material and are predominantly sequestered in an inactive form whithin a thick membrane in order to prevent free-access to cellular constituents. Many chemical, physical and environmental stressors, including pollutants, are known to destabilize lysosomal membranes and membrane damage is proportional to the magnitude of stress. Lysosomes are known to play a major role in the sequestration of organic contaminants including PAHs. Although the ability to sequester contaminants from sensitive intracellular site is an advantageous protective mechanism. It renders the lysosomal membrane particularly susceptible to elevated toxicant concentrations, leading to the efflux of hydrolytic enzymes and enhanced autophagic activity with a resultant diseased state.

The complex halogenated mixtures produced by pulp mills induce the monooxygenase enzyme system, which can be measured via several tests, including the induction of ethoxyresorufin O-deethylase (EROD). Receptor-mediated induction of cytochrome P450-dependent monooxygenase by xenobiotic chemicals has also provided a highly sensitive indicator of contaminant exposure. The suitability of various antioxidant parameters, such a glutathione S

Table 1. Various Biomarkers and pollutants.

Biomarkers	Effects	Class of pollutants
CYP1A1 expression (EROD)	induction	organochlorine pesticides, polychlorobiphenyls, dioxins, polycyclic aromatic hydrocarbons
Benzopyrene hydroxylase	induction	Benzo(a)pyrene
Glutathione-S-transferase	induction	Electrophilic substances
Acetylcholinesterase	inhibition	organophosphate and carbamate insecticides
Metallothionein synthesis	induction	metals
Stress proteins	induction	metals, other xenobiotics
DNA damage, DNA adducts	occurrence	mutagens, genotoxic xenobiotics
Acetylcholinesterase	inhibition	organophosphate and carbamate insecticides
Lipid peroxydation	induction	non-specific
Lysosomal system integrity and functioning	decrease	non-specific

transferase, superoxide dismutase, catalase, glutathione peroxidase, glutathione reductase, NADPH diaphorase, glutathione and lipid peroxidation, for use as biomarkers has been examined in a variety of organisms (Table 1).

Exposure to heavy metal ions can result in acute toxicity. In addition, exposure to certain heavy metals such as Cr, Ni, As and Cd can lead to carcinogenesis. Some protection against the toxic and carcinogenic effects of heavy metal ions is provided by the metallothioneins (MT), which are low molecular weight, cystein rich, heavy metal binding proteins. DNA binding will be used to study the interaction of these proteins with the MRE and determine which one of them is the sought-after metal response factor (MRF). The mechanism by which heavy metal ions control the activity of the MRF will be investigated using biochemical and reversed genetic approaches. The induction of metallothionein synthesis is typical of metal pollution. Metallothioneins are cysteine-rich, metal-binding proteins of a low molecular weight (> 10 kDa), whose syntheis is induced upon exposure to trace metals. These proteins regulate the uptake and tissue distribution of essential trace metals such as the transition elements zinc and copper. The induction of their synthesis is an adaptative and detoxifying mechanism, since these proteins covalently bind heavy metals and block their reactivity.

Approaches and Prospectives of Environmental Genomics

Gene expression research frequently is classified as either mechanistic or predictive. Mechanistic research focuses on how chemicals may affect genes, proteins and metabolic processes. Predictive studies seek to establish cause-effect relationships between events in the exposure-disease contiuum. Toxicogenetics focuses on individual genetic differences, while toxicogenomics tends to focus on general mechanisms of toxic response. While toxicogenetics focuses on the role of the individual genes in moderating or accentuating the effects of environmental agents, Environmental genomics measures thousands of genes and gene products to determine how environmental agents affect overall gene function.

Through recombinant DNA techniques, researchers can detect the environmentally induced changes in the structure and sequence of DNA indicated by biomarkers. Automated immunoassay technology currently in development can detect biomarkers of specific disease-causing compounds in biological fluids.

A biomarker is the result of a subclinical event in the body caused by exposure to an environmental factor, such as a chemical or radiation. It is an indicator of disease susceptibility. Ideally, the increased risk for disease associated with the presence of the biomarker should be reversible by appropriate medical intervention. Common sample materials for biomarker assays are blood, urine, feces, hair, and saliva. DNA samples are usually derived from blood cells, and the presence of chemical metabolites is usually determined from urine.

The mechanisms by which heavy metals exert their carcinogenic activities were studied. As there is little evidence that carcinogenic heavy metals such as As or Cd act as tumor initiators via a standard genotoxic mechanism, it might be suggested that there are tumor promoters by causing oxidative stress. Since tumor promotion by phorbol esters is known to be associated with increased expression of protooncogenes, the induction of these genes in response to heavy metal ions were studied.

A likely induction mechanism could be initiated with either the inhibition of tyrosine phosphatases or with the activation of tyrosine kinases by heavy metal induced oxidative stress. Activation of tyrosine kinas-

es triggers a signalling cascade that results in the stimulation of serine/threonine specific protein kinases including a protein kinase that phosphorylates and potentiates the activity of the cJun protein, leading to auto-stimulatory induction of gene expression. These studies should provide us with a molecular basis for understanding how certain heavy metal ions can act as tumor promoters and thereby exert their carcinogenic activity.

The cDNA microarrays are tools that can be used to analyze changes in genomewide patterns of gene expression. This technology may potentially revolutionize the way toxicological problems are investigated. The main challenges facing investigators in environmental health research is to assess exposures and identify hazards. Defining the mechanisms of action of environmental agents can greatly assist in hazard identification, species extrapolation, and risk assessment. Given that exposures to different classes of toxicants result in distinct patterns of altered gene expression, microarray technology can be utilized to categorize and classify these effects. In defined model systems, treatment with known agents, such as polycyclic aromatic hydrocarbons, dioxin-like compounds, peroxisome proliferators, oxidant stress, or estrogenic chemicals may provide a gene expression "signature" on a microarray which represents the cellular response to these agents. These same systems can then be treated with unknown, suspect agents to determine if one or more of these standard signatures is elicited. Currently the "DNA chips" are developed in human cDNA clone subarrays oriented toward the expression of genes involved in responses to xenobiotic metabolizing enzymes, cell cycle components, oncogenes, tumor suppressor genes, DNA repair genes, estrogen-responsive genes, oxidative stress genes, and genes known to be involved in apoptotic cell death.

Recently the effect of benzene exposure on peripheral blood mononuclear cell (PBMC) was studied about gene expression in a population of shoe-factory workers with occupational exposures using microarrays and real-time PCR. PBMC RNA was stabilized in the field and analyzed using a human array. The microarrays could identify changes in gene expression that could be used as new biomarkers of exposure and early effect for benzene and provide information on mechanisms of benzene toxicity. The overall goal is to provide potential gene markers of exposure and early effect for benzene and to produce mechanistic insight into how benzene affects the body, especially the immune system and lymphocyte function. Gene expression profiles of interest were significantly up-regulated or down-regulated in experimental group when compared with control group. Genes showing highly altered experession levels were aligned in the order of magnitude of altered expression in experimental group. Gene expression profiles showed that 16 genes were up-regulated in experimental group. Those are chemokine receptor, Bruton

Table 2. Recent Environmental Genomics in Korea.

Species	Microarray	Pollutants	Regulated genes
Human	416 genes	EDC (estradiol, progesterone, testosterone, alkylphenol, phthalates	Cytochorme P450 1B1, protein p53 Thyroid hormone receptor etc.
Human	8 K	methyl mercury	phosphotidylinositol transferase, 90 kDa-heat shock protein, nucleosome assembly protein etc.
Rat	490 genes	2,3,7,8-tetrachlorodi benzo-p-dioxin	Transcription factor, metallothionein, cytochrome P450 subfamily, diaphorase, carnitine palmitoyltransferase, Glutathione-S-transferase etc.
Rat	4.8 K	Thioacetamide	Serine proteinase inhibitor, cathepsin B, Thioredoxin etc.
Mouse	7.4 K	Carbontetrachloride	Plexin B1, glutamate-cysteine ligase, catalytic subunit, cytochrome P450 subfamily etc.
Rat	3.5 K	Arsenic	Mitogen-activated protein kinase, heme oxygenase, glutathione S-transferase etc.
Oryzias Latipes	3.4 K	Nonylphenol	Vitellogenin, choriogenin etc.
medaka	2 K	17-β-estradiol	Estrogenic potential related genes
Mouse	35 K oligo	Vinyl chlolide, aldrin, 2,4,5-phenoxycaetatic acid, copper sulfate	Urinoglobulin, Plasminogen activator, F11coagulation factor XI, cysteine proteinase etc.

agammaglobulinemia tyrosine kinase, G1/S-specific cyclin D3, Mitochondrial creatinekinase gene, Thyroid stimulating hormone receptor, Proteinkinase, Src kinase-associated phosphoprotein of 55 kDa, Mitogen-activated protein kinase, Ephrin, Phosphatidylinositol-4-phosphate-5-kinase type II beta, NF-E2 related factor, Protein phosphatase 2-regulatory subunit B, Calmodulin 3 (phosphorylase kinase, delta), Lectin, galactoside-binding, soluble, LIMdomain kinase, Transcriptional factor II. Gene expression profiles showed that 36 genes were down-regulated in experimental group. Those are tumor necrosis factor receptor superfamily, dual specificity tyrosine phosphorylation regulated kinase, protein phosphatase, Bcl-2-related protein A.

In Korea, there are several research laboratories for kicking off these Environmental Genomics as shown in Table 2.

Validated molecular biomarkers have long been recognized as invaluable tools for identifying and preventing human disease. The potential of molecular biomarkers is especially high in relation to preventing environmentally induced disease. This is an important focus of health research at present because of significant concern over the risk of human exposure to persistent organic pollutants, heavy metals, airborne pollutants and environmental agents. Molecular biomarkers could play a key role in facilitating advances in disease detection and prevention.

A useful biomarker is highly accurate and reproducible, characteristics that may be thoroughly established during the development phase of the marker using statistical methods and laboratory studies. Internal controls are extremely valuable for ascertaining accuracy and reproducibility. Controls should also be used to characterize and quantify the dynamic range of the assay.

Biomarkers are powerful tools because they can identify and quantify exposure, effect, or susceptibility in individual members of a population. Thus a biomarker must be sufficiently sensitive to provide an accurate measurement in a sample of limited quantity from a single individual. Sensitivity and specificity are inversely related to one another. Specificity must be sufficient to avoid a high false positive rate and sensitivity must be sufficient to avoid a high false negative rate. These complications include variable rates of carcinogen activation and adduct repair and the validity of using surrogate tissue in place of the target tissue. Anyhow, Environmental genomics will be a great promising next generation technology in the fields of epidemiology and environmental health risk assessment.

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