The Use of Blood Cell Gene Expression Profiles as a Surrogates for Target Organ Toxicity

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Abstract
Microarray analysis of mRNA changes due to up-regulated and down-regulated polymorphonulear leucocyte (PMNL) gene responses to toxicant exposure have been proposed to permit the assessment of toxicant visceral tissue effects and gene responses in rodent models and human exposure situations. The use of PMNL as surrogates for toxicant target organs such as lung, brain and liver has been used to develop gene expression profiles characterizing toxicant exposure capable of also monitoring subclinical and clinical toxicant exposures.
Although interpretation of gene expression data can be confounded by difficulties such as direct toxicant PMNL effects, test animal stress responses. Inflammatory effects of inappropriately large toxicant doses, the use of PMNL gene expression responses as surrogates of visceral tissue toxicant effects, show promise as a toxicological tool to monitor as well as characterize genomic response to toxicant exposure.

Acute and chronic xenobiotic exposures have been shown to produce characteristic alterations in gene expression [1] which can be utilized to study mechanisms of toxicity and predict or monitor potential human adverse effects .The investigation of genetic response to toxicants [2] Potential human and environmental toxicants, and their putative mechanisms of action can be identified through the use of genomics resources [3] such as DNA microarray analysis following recovery of RNA from peripheral blood polymorphonuclear leukocytes (PMNL) used as surrogates for toxicant target tissue enabling the simultaneous monitoring of the expression levels of up-regulated, unchanged or down-regulated genes. Results obtained permit the development of gene expression profiles (GEP) which have promise as biomarkers for toxicity [3,4.]
In response to toxicant exposure, target and surrogate tissue gene expression is altered prior to and/or during subclinical or clinical toxicity, The determination of the characteristic and specific pattern of gene expression elicited by a given toxicant on PMNL can permit an assessment of toxicant exposure on human visceral toxicant target tissue which may not be readily available. However the analysis of tissue surrogate response such as blood gene expression can be used to detect the onset of target organ toxicity [4] Circulating PMNL constitute readily available surrogate tissue from which the study of mRNA changes due to xenobiotic perturbation of a multitude of genes indicates gene expression effects (up regulation or down regulation) resulting from an in vivo target tissue chemical exposure at a specified dose and duration [5-7].
The objective of the present communication is to acquaint the reader with recent research illustrating the use of surrogate tissue to profile differential gene expression changes induced by toxicants targeting cardiac (8) neuronal [4,9] endocrine [24] hepatic [10-13]; and pulmonary tissue [14-19]

Although pulmonary function tests and chest x-ray can monitor the onset of silicosis in humans, the measurement of GEP in easily available surrogate tissue such as blood during or following toxicant exposure can permit the detection of preclinical toxicity before irreversible toxicity develops. Differential gene expression in PMNL from test subjects exposed to toxicants, have been used to characterize blood GEP to monitor the development of silica pulmonary toxicity [16], adriamycin cardiotoxicity [8], acetaminophen hepatotoxicity environmental estrogen exposure [24] and methylparathion neurotoxicity [4] in rat models of human toxicant exposure.

Three month old male Fisher rats were exposed to inhaled crystalline silica for 5 days while pulmonary damage and GEP of PMNL was measured up to 16 weeks to model occupational silica exposure and development of silicosis [16] Levels of inflammatory cytokines, alveolar macrophages, PMNL in brochoalveolar lavage fluid and lung histopathology were correlated with blood PMNL and GEP data. Compared with time-matched controls, a prediction signature of upregulated genes from the blood of silica exposed animals was used to identify rats exposed to three different silica concentrations.

Differential gene expression was observed in blood and cardiac samples from adriamycin treated male rats which was correlated with their histopathology, A large number of genes were up and down regulated in blood and heart tissue by adriamycin treatment consistent with the known toxicity of this antineoplastic agent [8]. Up regulated genes included those coding for NADPH oxidoreductase, heme oxygenase 1, and IL-1 receptor antagonist protein precursor. However inconsistent blood and heart GEP data was obtained for Mn SOD, iNOS and Glutathione S transferase Ya subunit genes. In some instances cardiac tissue gene expression was down regulated while that of blood genes were up regulated. This data illustrates several confounding effects of these studies: For example, the subjects were obviously stressed due to GI toxicity, impairment of dietary intake, local tissue toxicity at the injection site and inflammatory responses as well as potential direct effects of the toxicant on the PMNL.
Male Fischer 344 rats were given single acute doses of acetaminophen or methyl parathion in order to determine whether their effect on blood GEP changes reflected liver or brain toxicity respectively [4]. Elevations in blood transaminase and inhibition of blood acetylcholinesterase provided evidence of hepatotoxicity and neurotoxicity respectively. Twelve hepatotoxicity and neurotoxicity marker genes were down regulated in the blood of treated animals. Direct *In vitro* effects may confound the interpretation of the observed results since *in vitro* exposure of Leucocytes produced increased expression of four hepatotoxicity marker genes. The expression of one marker gene (Ddah 1) was increased more than twice that of the corresponding hepatic tissue marker gene. The data indicate that *In vitro* toxicant GEP effects [6] can contribute to the observed results thereby confounding their interpretation Furthermore, the relationship of the toxicant concentration used *in vitro* to that present *in vivo* was not determined. Of the 31 candidate hepatotoxicity marker gene evaluated, 11 were found to be specific for liver toxicity. i.e., they were differentially expressed in blood samples of rats treated with different liver toxicants while not changing their expression in animals treated with the neurotoxicants.

In contrast, fewer neurotoxicity marker genes exhibited comparable specificity to the hepatotoxicity marker genes, Similarly, perpheral blood lymphocytes and livers from adult male Wistar rats treated with single ethanol doses or five daily phenobarbital or 3-methycholantrene doses exhibited increased expression of genes involved in phase I and phase II xenobiotic metabolism. apoptosis and inflammation [5] indicating that following xenobiotic exposure these genes are expressed in polymorphonuclear leukocytes and in liver tissue [11].

Blood gene expression profiles were shown to be capable of distinguishing toxic from non toxic dose acetaminophen exposed rats with greater accuracy than predictions based on histopathology, hematology or clinical chemistry data [10]

Surrogate Blood Gene Expression Profile Use in Human Toxicant Exposure Aalthough the use of surrogate GEP monitoring of animal models suggests that this may be of use in monitoring human exposure, a limited number of studies involving intentional toxicant exposure of human subjects have been reported to date. [17,18]. However blood cell differential gene expression has been reported in human environmental exposures to tetrachloro-dibenzo-p-dioxin (TCCD) polychlorinated biphenyl [20-22] tobacco smoke [15], diesel [18, 19] and metal fumes

Tobacco Smoke

Lymphocyte GEP in a large number of human subjects consisting of 297 current smokers was determined [15]. Plasma levels of the nicotine metabolite, cotinine provided a quantal characterization of smoking activity. Smoking correlated genes included many related to lymphocyte proliferation, inflammatory response, lung- related cardiovascular disorder, free radial scavenging and xenobiotic biotransformation. Expression profile of up and down-regulated genes from blood of non smoking, former smokers, recent and current smokers correlated with their smoking status and blood levels of cotinine [14]. However *in vitro* blood mononuclear cell exposure to cigarette smoke condensate has been shown to also alter GEP [25] suggesting that direct tobacco smoke effects may contribute to the results observed *in vivo*.

Diesel Exhaust Fumes

Intentional inhalational exposure of a small number of healthy males to diesel exhaust produced peripheral blood mononuclear cell GEP changes involving nine genes associated with oxidative stress, protein degradation and coagulation [19] A minority of peripheral blood mononuclear genes were found to be up-regulated in human volunteers exposed for 2 hrs to diesel exhaust compared with those exposed to fresh air [18] These included genes involved in oxidative stress , leukocyte activation, cell adhesion, migration and inflammation.

Environmental Toxicant Exposure

In children with environmental polychlorohydrocarbon exposure blood GEP comparisons between high and low level contaminated individuals revealed twelve differentially regulated genes associated with skeletal muscle, genetic and connective tissue and neurological disorders [20, 22] Global mononuclear cell gene expression 20 yrs after Tetrachlorodibibenzo-p-dioxin (TCDD) exposure indicated differential gene expression between subjects with high and low TCDD blood levels [21]. A majority of genes exhibited less than 29% up or down-regulation. Twenty two genes were classified as up- regulated while approximately 113 were down-regulated by dioxin exposure. Peripheral mononuclear cell differential up regulated hemoglobin gene expression in subjects exhibiting chloracne (a dermal manifestation of dioxin exposure) differed from those without chloracne. In these subjects nine genes were found to be down regulated. The functional significance of these gene expression changes remains to be determined.

Metal Exposure

Short term occupational exposure to metal fumes was shown to produce small expression changes in genes related to proinflammatory and immune responses, apoptosis, phosphate metabolism, cell proliferation and oxidative stress [17]

A multitude of expression changes were observed in non smokers exposed to these fumes suggesting that tolerance may account for the lower GEP effects observed in smokers exposed to metal fumes. The observed differential up-regulation of inflammatory response genes in peripheral blood lymphocytes from arsenic-exposed individuals has been suggested to contribute to the increased risk of vascular ischemic disease [26]. Thus up- regulation of blood PMNL genes involved in inflammatory responses, constitutes the most predictable response to irritants in diesel, tobacco smoke and metal toxicants.

Conclusion

The measurement of blood PMNL gene transcription changes associated with xenobiotic exposures in humans and animal models of human exposure shows promise as an investigative and exposure monitoring tool. However the relevance of most human and animal PMNL GEP changes associated with xenobiotic exposures to toxic situations is not yet always clear. The inclusion in animal exposure models of dose response and time course studies as well as determinations of potential direct xenobiotic effects can be expected to increase their relevance to human exposure situations

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