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Systems Approach to Identify Environmental Exposures Contributing to Organ-Specific Carcinogenesis

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Abstract

The most effective way to reduce cancer burden is prevention which is dependent on identifying individuals at risk for a particular cancer and counselling them to avoid exposure to causative agents. Other than a few well characterized environmental agents linked to specific cancers, linkage between any particular environmental exposure and a specific type of cancer is mostly unknown. Thus, we propose a systems approach to analyse publicly available large datasets to identify candidate agents that play a role in organ-specific carcinogenesis. We have tested this approach using publicly available datasets for mRNA and miRNA expression in ovarian cancer to define the differentially expressed genes that are also targets of differentially expressed miRNAs. These target genes were then used to query the Comparative Toxicogenomics Database to identify interacting chemicals and also were analysed by Ingenuity Pathway Analysis to identify pathways. The results show that the interacting chemicals interact with genes in known pathways in ovarian carcinogenesis and support the hypothesis that these chemicals are likely etiologic agents in ovarian carcinogenesis. Thus, a systems approach may prove useful to identify specific etiologic agents to better develop personalized preventive medicine strategies for those most at risk.

Introduction

It is clear that carcinogenesis is driven by mutations and epigenetic changes in critical genes regulating cell replication and maintenance of genome integrity [12]. What is less clear are the specific environmental agents inducing oncogenic changes driving most cancers.

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Sometimes the oncogenic changes are epigenetic with changes in DNA methylation patterns playing a role in gene inactivation and activation [20]. More recently, changes in microRNA expression have been identified as playing a role in carcinogenesis [10;11;20;23].

Environmental agents that act as epimutagens have been identified [1;4;7;17], but not yet causally linked to specific cancers. The agents driving mutations in some cancers are well understood. For instance, ultraviolet light clearly is the environmental agent causing DNA damage resulting in mutations driving most skin cancers [5]. Likewise, carcinogens in tobacco smoke are known drivers of the mutations in lung cancers [13]. However, for many other tumors, the etiologic agents are unknown.

Identification of specific environmental exposures linked to organ-specific carcinogenesis can inform efforts to prevent those cancers from developing, especially in genetically susceptible individuals. Thus, development of ways to identify candidate exposures for further study and confirmation of linkage is vital for individualized preventive medicine [19].

We propose here that available large data sets provide the means to identify candidate agents for study of causality. Identifying environmental exposures that may contribute to organ-specific carcinogenesis would provide the basis for epidemiological studies to determine the causal relationships.

Environmental exposures are capable of inducing epigenetic changes, including changes in microRNA expression, that result in differential gene expression [6;14;16]. Most importantly, changes in microRNA expression may cause permanent changes in regulation of gene expression patterns leading to altered cellular phenotypes. These alterations in microRNAs represent dysregulation of gene expression in the cancer cells and may be a manifestation of environmental exposures [6]. Thus, we hypothesize that differential expression of microRNAs in a cancer are a consequence of carcinogenic environmental exposure and drive gene expression changes resulting from these environmental exposures. Hence, the cancer stem cell phenotype may arise as a consequence of environmental exposure driven changes in gene expression whether these changes are the consequence of mutation or epigenetic modification. We propose that a systems approach utilizing publicly available data for differential microRNA expression associated with a cancer and for differential mRNA expression in that cancer in conjunction with known associations between chemical exposures and gene expression will identify chemicals that play a role in carcinogenesis. We demonstrate the potential for this approach to identifying environmental contributions to development of a specific class of cancers using ovarian cancer (OvCa) as an example.

Other than an association of a small fraction with inherited mutations in BRCA1 and BRCA2 [19], most cases of OvCa are sporadic with cause unknown [15]. Using publicly available datasets, we identified both mRNAs and microRNAs differentially expressed in ovarian cancers. Intersection of genes with differential expression in ovarian cancer and putative gene targets of differentially expressed microRNAs in ovarian carcinomas gave a list of candidate genes for chemical interactions. Ingenuity Pathway analyses of these genes show that they participate in pathways involved in cancer and other chronic diseases. This

list of genes was submitted to the Comparative Toxicogenomics Database (CTD) and a list of chemicals with known interactions with these genes was collected. The list of chemicals interacting with the OvCa specific genes included known carcinogens (arsenic, asbestos, benzo[a]pyrene), co-carcinogens (tetrachlorodibenzodioxin, tetradecanoylphorbol acetate), peroxides, heavy metals (Cd, Cu, Zn), epigenotoxicants (hydralazine, valproic acid), inflammation inducers (zymosan, lipopolysaccharide), steroid hormones (estradiol, progesterone, dexamethasone), dietary chemopreventives (indole-3-carbinol, curcumin) and chemotherapeutics (doxorubicin, tamoxifen, tretinoin). While some of these chemicals and pathways have been implicated previously in ovarian carcinogenesis, (for instance, asbestos [18], tetrachlorodibenzodioxin [9]) many have not, and their identification will enable the development of targeted epidemiological studies to determine the role of exposure to these chemicals in ovarian carcinogenesis. This systems approach will likely be useful to identify environmental exposures contributing to cancer of other organs with unknown etiologies providing information essential to personalized preventive approaches to reducing cancer incidence.

Materials and methods

Genes differentially expressed in ovarian cancer compared to normal ovary tissue were determined using publicly available data sets. The two data sets of gene expression used in this study were as follows. Normal ovary gene expression was extracted from NCBI Gene Expression Omnibus (GEO) GSE1133. This data set contains baseline mRNA abundance of various human and mouse tissues. The human ovary data were assayed by two Affymetrix chip platforms: U95 and U133. Ovarian tumor gene expression was extracted from NCBI GEO GSE6822. This data set contains human ovarian cancer gene expression data assayed by the Affymetrix Hu6800 platform from a large heterogeneous set of 74 ovarian tumors. The Affymetrix U95 platform assays 8,075 genes. The company provides a list of good matches between U95 and U133 platforms. From this list we extracted 5,804 genes as being present in both platforms.

We used Perl scripts to match the three platforms, and to extract genes that were present on all three platforms. The p-values of differential expression between healthy ovaries and ovarian cancer were calculated with the regularized t-test [2], and the Benjamini and Hochberg correction [3] was applied to control the false discovery rate. After analysis of significance, 723 genes (Set A) were designated as differentially expressed between ovary and ovarian cancer.

The microRNAs differentially expressed in ovarian cancer cells identified by Wyman et al [22] were used to query the Sanger miRBase for target mRNAs. Wyman et al identified 56 differentially expressed microRNAs. The Sanger miRBase lists 3,916 mRNAs (Set B) as targets of these 56 microRNAs. The intersection of Sets A and B has 380 genes (Set C).

Next we took the intersection of Set A and Set B, and obtained 380 genes. These 380 differentially expressed mRNA targets of differentially expressed microRNAs were designated gene Set C. Set C was submitted to the Comparative Toxicogenomics Database (CTD, <http://ctdbase.org/>, [8]) to find the known interactions of these genes with chemicals.

The CTD is a curated database compiling associations between gene expression, chemical exposure and disease data reported in the literature. The interacting chemicals were ranked by their numbers of gene interactions. Lastly, Set C was analysed for gene interaction networks and pathways with Ingenuity Pathway Analysis software.

Results

The 380 genes identified as differentially expressed in ovarian cancer and also as targets of microRNAs differentially expressed in ovarian cancer are listed in Figure 1A. The majority of the 380 genes had decreased expression (Figure 1A, green) in ovarian cancer cells with only 99 showing increased expression (Figure 1A, red).

Query of the CTD with the 380 differentially expressed genes that also were targets of differentially expressed microRNAs in ovarian cancer cells (Figure 1A) identified 889 chemicals with known interactions with these genes. Some genes had interactions with many chemicals, some with as few as one. Likewise, some chemicals had interactions with a large number of genes, and some with as few as one. The number of genes interacting with each chemical ranged from 1 to 162 as shown in Figure 1B.

We arbitrarily reduced the set to the 110 chemicals interacting with at least 9 genes to show in Table 1. Among these chemicals are known carcinogens, heavy metals, steroid hormones, chemopreventives, and cancer chemotherapeutics, to name just a few of the classes. It should be noted that some of the listings are different forms of the same active agent, e.g. arsenic is listed also as sodium arsenite and arsenic trioxide.

We next performed network analysis of the genes with differential expression in ovarian cancer cells using IPA. The top three networks are shown in Figures 2–4. Included in these networks are chemical interactions and functional pathways in the IPA knowledge base. Most of the chemicals identified as interacting with genes in these networks by IPA are found in the list of chemicals identified by the CTD analysis and include PAH, arsenicals, asbestos, cadmium, dioxin and PCBs. These networks include ones involved with tissue morphology, cancer and nucleic acid metabolism. The top network (Hematological System Development and Function, Humoral Immune Response, Tissue Morphology; Figure 2) includes genes in three ovarian cancer subnetworks: “Response to therapy” (platelet derived growth factor complex [PDGF], chemokine (c-c motif) ligand 2 [CCL2]), “Prognosis” (CCL2, FBJ murine osteosarcoma viral oncogene homolog B [FOSB], nuclear factor kappa B [NFkB]), and “Diagnosis” (Laminin, keratin 19 [KRT19], mucin 1 [MUC1], interleukin 12 [IL12], CCL2).

Most intriguing are the interactions with an ovarian cancer signaling pathway in the Cancer, Genetic Disorder, Renal and Urological Disease network (Figure 3). This signaling pathway includes the genes transcription factor 3 [TCF3], mitogen-activated protein kinase 3/ mitogen-activated protein kinase 1 [MAPK, ERK1/2, ERK], tumor protein 53 [TP53], v-akt murine thymoma viral oncogene homolog 1 [AKT], v-akt murine thymoma viral oncogene homolog 2 [AKT2], and catenin [CTNNB].

The third network is DNA Replication, Recombination, and Repair, Molecule Transport, Nucleic Acid Metabolism. This network includes subnetworks for developmental process of cancer, arrest in cell division process, inflammatory disorder of organ, damage of DNA and tumorigenesis of mammalia. These subnetworks all overlap with one another with major hubs on tumor necrosis factor [TNF] and interferon gamma [IFNG], and minor hubs on promyelocytic leukemia [PML], retinoic acid receptor alpha [RARA], interleukin 5 [IL5], NFkB1, insulin 1 [INS1], and 'lectin, galactoside-binding, soluble, 12' [LGAL12].

Discussion

We have used a systems approach to identify environmental agents that may play a role in ovarian carcinogenesis. Our analysis yielded a number of candidates for further study including a number of known carcinogens, an endocrine disruptor, a pesticide, heavy metals, inflammatory agents and aldehydes implicated in oxidative damage. The vast majority of these agents has not been previously linked to ovarian cancer thus highlighting the ability of this systems approach to identify unsuspected agents as candidate ovarian carcinogens. The network analyses (Figures 2–4) show known associations of representatives of these classes of carcinogenic chemicals with genes differentially expressed in ovarian cancer. In all three top networks, carcinogens interacting with genes differentially expressed in ovarian cancer include arsenic in several forms, asbestos, benzo[a]pyrene and its metabolites, cadmium and cadmium salts, dioxins and polychlorinated biphenyls. Some of these chemicals have been classified as ovarian carcinogens, whereas others have been suspected but clear evidence has not yet been found as discussed in the following paragraphs.

Arsenic is a well known carcinogen and arsenic exposure is causally linked to lung, skin, liver and kidney cancers. Arsenic exposure *in utero* induces ovarian cancer in mice and the incidence of cancers is increased when that exposure is continued after birth [5]. These observations suggest that investigating the association between arsenic exposure and ovarian cancer in humans is warranted.

Asbestos exposure is causally linked to mesothelioma [6] and most studies of asbestos in relation to carcinogenesis have been focused on lung cancer. Asbestos exposure augments TNF expression in macrophages as a consequence of activation of the dual specificity MAPK phosphatase (DUSP6, aka MKP3) [7]. It was speculated that asbestos might contribute to ovarian cancer in women who used talcum powder in the genital area [8]. Data from a recent meta-analysis support the hypothesis that occupational exposure to asbestos is associated with increased incidence of ovarian cancer [9].

Benzo[a]pyrene is the most common environmental chemical carcinogen and is a product of incomplete combustion of organic materials. A major source of benzo[a]pyrene exposure is tobacco smoke. Tobacco smoke has been linked to a variety of human cancers, most notably lung cancer. However, it is widely recognized that tobacco use causes over 30% of human cancer deaths [10]. Increased risk of mucinous ovarian cancer in smokers [11] was sufficient evidence for the IARC to classify cigarette smoking as a cause of ovarian cancer [12]. However, a large meta-analysis suggests that this increase may be offset by decreases in endometrioid and clear-cell ovarian cancers [13]. Thus, it appears that smoking, and by

extrapolation PAH exposure, may play a role in specific histological subtypes of ovarian cancer. Our analysis did not stratify ovarian cancers by subtype, yet it found the relationship of PAH and ovarian cancer. Thus, although PAH are not the only carcinogens in cigarette smoke, the power of the systems approach is demonstrated and the observations suggest that more detailed examination of the causal relationship of cigarette smoking and other means of PAH exposure to ovarian cancer be performed.

IARC classifies cadmium and cadmium compounds as carcinogenic in humans (Group 1) [14]. Inhaled cadmium clearly causes lung cancer [14]. Cadmium also likely causes prostate cancer in humans [14]. The evidence for a role of cadmium exposure in ovarian carcinogenesis is not so clear. An association of urinary cadmium with mortality from ovarian cancer was observed in an analysis of the third NHANES cohort [15]. However, no association between dietary cadmium and ovarian cancer was observed in a Swedish cohort [16]. Our analyses linked cadmium to genes in the ovarian cancer signalling network (Figure 3). It is possible that the dietary exposures in the Swedish cohort were insufficient to show a significant association whereas occupational exposures may show a significant correlation.

Dioxin exposure has been studied mostly in terms of liver cancer. However, ovarian tumors can be induced in rats by chronic treatment with dioxin [17] and one study found ovarian cancers in an Italian population exposed to dioxin in childhood after an industrial accident [18]. Similar to dioxins, PCBs have been studied mostly in relation to liver disease. An association of ovarian cancer with exposure to polychlorinated biphenyls has not been reported. Our observations suggest that further investigation of the relationship of dioxin, PCB and organochlorine exposure and ovarian cancer is warranted.

The systems approach used in the present study demonstrated linkage between known interactions of several carcinogens with genes playing a role in ovarian cancer signalling. This linkage supports the likelihood that these environmental agents play a role in ovarian carcinogenesis. A few of these carcinogens have been implicated in ovarian carcinogenesis in rodents and/or humans, further validating the approach. The robustness of this approach is supported by the fact that the mRNA and microRNA data sets used in the analysis are derived from completely different ovarian cancer sample sets. In addition to the few chemicals known or suspected to play a role in ovarian carcinogenesis, a number of other carcinogens and hormonal agents not previously suspected of playing a role in ovarian carcinogenesis were identified as having linkages to microRNA target genes differentially expressed in ovarian cancer.

The mechanism of the effect of these newly identified agents on ovarian carcinogenesis might be directly related to higher exposure associated with specific occupations, or might be more indirect, requiring interaction with other environmental or genetic factors. It is possible that any role in ovarian carcinogenesis may be limited to specific histological subtypes. Clearly, further research into the part these agents may play in ovarian carcinogenesis, and especially in women with known genetic susceptibility genes, is warranted.

A similar systems approach is likely to prove useful in identifying environmental agents playing etiologic roles in carcinogenesis of other organs. This approach using existing databases is limited by the available information relating differential gene expression with exposure to specific agents. However, these databases are continually updated providing additional information with each update. Thus, the candidates identified at a particular time are likely a partial list of contributing factors. As our understanding of gene expression networks and how these networks are perturbed by chemical exposures evolves, exposures leading to specific disease states, in particular, specific cancers and their subtypes will be identified leading to a more holistic approach to cancer prevention and treatment [21].

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References

1. Baccarelli A, Bollati V. Epigenetics and environmental chemicals. *Curr Opin Pediatr*. 2009; 21:243–251. [PubMed: 19663042]
2. Baldi P, Long AD. A Bayesian framework for the analysis of microarray expression data: regularized t-test and statistical inferences of gene changes. *Bioinformatics*. 2001; 17:509–519. [PubMed: 11395427]
3. Benjamini, Yi; Hochberg, Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Royal Statistical Society, Series B*. 1995; 57:125–133.
4. Bhattacharjee P, Banerjee M, Giri AK. Role of genomic instability in arsenic-induced carcinogenicity. A review. *Environ Int*. 2013; 53:29–40. [PubMed: 23314041]
5. Brash DE. Sunlight and the onset of skin cancer. *Trends Genet*. 1997; 13:410–414. [PubMed: 9351343]
6. Chen T. The role of MicroRNA in chemical carcinogenesis. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev*. 2010; 28:89–124. [PubMed: 20552498]
7. Collotta M, Bertazzi PA, Bollati V. Epigenetics and pesticides. *Toxicology*. 2013; 307:35–41. [PubMed: 23380243]
8. Davis AP, Murphy CG, Saraceni-Richards CA, Rosenstein MC, Wieggers TC, Mattingly CJ. Comparative Toxicogenomics Database: a knowledgebase and discovery tool for chemical-gene-disease networks. *Nucleic Acids Res*. 2009; 37:D786–D792. [PubMed: 18782832]
9. Davis BJ, McCurdy EA, Miller BD, Lucier GW, Tritscher AM. Ovarian tumors in rats induced by chronic 2,3,7,8-tetrachlorodibenzo-p-dioxin treatment. *Cancer Res*. 2000; 60:5414–5419. [PubMed: 11034082]
10. Di LG, Croce CM. miRNA profiling of cancer. *Curr Opin Genet Dev*. 2013; 23:3–11. [PubMed: 23465882]
11. Di LG, Croce CM. The Role of microRNAs in the Tumorigenesis of Ovarian Cancer. *Front Oncol*. 2013; 3:153. [PubMed: 23785667]
12. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell*. 2011; 144:646–674. [PubMed: 21376230]
13. Hecht SS. Lung carcinogenesis by tobacco smoke. *Int J Cancer*. 2012; 131:2724–2732. [PubMed: 22945513]
14. Iorio MV, Croce CM. Causes and consequences of microRNA dysregulation. *Cancer J*. 2012; 18:215–222. [PubMed: 22647357]
15. Kurman RJ, Shih I. Molecular pathogenesis and extraovarian origin of epithelial ovarian cancer--shifting the paradigm. *Hum Pathol*. 2011; 42:918–931. [PubMed: 21683865]

16. Lovat F, Valeri N, Croce CM. MicroRNAs in the pathogenesis of cancer. *Semin Oncol*. 2011; 38:724–733. [PubMed: 22082758]
17. Reichard JF, Puga A. Effects of arsenic exposure on DNA methylation and epigenetic gene regulation. *Epigenomics*. 2010; 2:87–104. [PubMed: 20514360]
18. Reid A, de KN, Musk AW. Does exposure to asbestos cause ovarian cancer? A systematic literature review and meta-analysis. *Cancer Epidemiol Biomarkers Prev*. 2011; 20:1287–1295. [PubMed: 21610219]
19. Shanmughapriya S, Nachiappan V, Natarajaseenivasan K. BRCA1 and BRCA2 mutations in the ovarian cancer population across race and ethnicity: special reference to Asia. *Oncology*. 2013; 84:226–232. [PubMed: 23364291]
20. Suzuki H, Maruyama R, Yamamoto E, Kai M. Epigenetic alteration and microRNA dysregulation in cancer. *Front Genet*. 2013; 4:258. [PubMed: 24348513]
21. Tian Q, Price ND, Hood L. Systems cancer medicine: towards realization of predictive, preventive, personalized and participatory (P4) medicine. *J Intern Med*. 2012; 271:111–121. [PubMed: 22142401]
22. Wyman SK, Parkin RK, Mitchell PS, Fritz BR, O'Briant K, Godwin AK, Urban N, Drescher CW, Knudsen BS, Tewari M. Repertoire of microRNAs in epithelial ovarian cancer as determined by next generation sequencing of small RNA cDNA libraries. *PLoS One*. 2009; 4:e5311. [PubMed: 19390579]
23. Yanaihara N, Harris CC. MicroRNA Involvement in Human Cancers. *Clin Chem*. 2013; 59:1811–1812. [PubMed: 23462030]

Figure 1. Differentially expressed genes that are targets of differentially expressed microRNAs (A). Interaction map of chemicals with genes differentially expressed in ovarian cancer. Induced genes in red; suppressed genes in green (B).

Figure 2. Differentially expressed microRNA target genes, Network 1 showing chemical interactions and functional pathways. Gene color code: red, induced expression; green, suppressed expression; yellow, multiple probe sets on chips with both induced and suppressed expression; blue, inserted by IPA to link genes in network.

Figure 3. Differentially expressed microRNA target genes, Network 2 showing chemical interactions and functional pathways. Gene color code: red, induced expression; green, suppressed expression; yellow, multiple probe sets on chips with both induced and suppressed expression; blue, inserted by IPA to link genes in network.

Figure 4. Differentially expressed microRNA target genes, Network 3 showing chemical interactions and functional pathways. Gene color code: red, induced expression; green, suppressed expression; yellow, multiple probe sets on chips with both induced and suppressed expression; blue, inserted by IPA to link genes in network. Signaling molecules inserted by IPA to link genes in network in purple.

Table 1

Chemicals interacting with differentially expressed genes that are targets of differentially expressed microRNAs

<u>Chemical name</u>	<u>Classification</u>	<u># gene interactions</u>
4-hydroxy-2-nonenal	aldehyde	15
Acrolein	aldehyde	10
Cycloheximide	antibiotic	19
Dactinomycin	antibiotic	9
Trichostatin A	antifungal	14
Ibuprofen	anti-inflammatory	41
Indomethacin	anti-inflammatory	32
Aspirin	anti-inflammatory	22
Tripterine	anti-inflammatory	16
Celecoxib	anti-inflammatory	10
Quercetin	antioxidant	15
Flavonoids	antioxidant	13
R 848	antiviral	10
3-(2-hydroxy-4-(1,1-dimethylheptyl)phenyl)-4-(3-hydroxypropyl)cyclohexanol	cannabinoid	10
Tetrachlorodibenzodioxin	carcinogen	61
Sodium arsenite	carcinogen	40
Benzo(a)pyrene	carcinogen	37
Asbestos, Crocidolite	carcinogen	33
Nickel sulfate	carcinogen	17
Methylcholanthrene	carcinogen	13
Arsenic	carcinogen	13
Benzene	carcinogen	12
Cadmium Chloride	carcinogen	12
2-Naphthylamine	carcinogen	10
Chlorodiphenyl (54% Chlorine)	carcinogen	10
N,N,N',N'-tetrakis(2-pyridylmethyl)ethylenediamine	chelator	13
Resveratrol	chemopreventive	105
Indole-3-carbinol	chemopreventive	25
Genistein	chemopreventive	25
Polyphenols	chemopreventive	11
N-(2-cyclohexyloxy-4-nitrophenyl)methanesulfonamide	chemopreventive	10
Doxorubicin	chemotherapeutic	68
Fluorouracil	chemotherapeutic	60
Arsenic trioxide	chemotherapeutic	41
Tamoxifen	chemotherapeutic	25
Irinotecan	chemotherapeutic	24
Cisplatin	chemotherapeutic	24
Sulindac sulfide	chemotherapeutic	24

<u>Chemical name</u>	<u>Classification</u>	<u># gene interactions</u>
Sulindac	chemotherapeutic	23
Carmustine	chemotherapeutic	21
Methotrexate	chemotherapeutic	17
Paclitaxel	chemotherapeutic	16
Ionafarnib	chemotherapeutic	16
Fulvestrant	chemotherapeutic	14
Etoposide	chemotherapeutic	13
Decitabine	chemotherapeutic	13
Thalidomide	chemotherapeutic	10
Oxaliplatin	chemotherapeutic	10
N(1),N(11)-diethylnorspermine	chemotherapeutic	10
Mitomycin	chemotherapeutic	10
Hydroxytamoxifen	chemotherapeutic	9
Drugs, Chinese Herbal	chemotherapeutic	9
Raloxifene	Selective Estrogen Receptor Modulator	24
Bisphenol A	endocrine disruptor	9
Zinc	heavy metal	44
Copper	heavy metal	30
Iron	heavy metal	17
Uranyl acetate	heavy metal	13
Uranium	heavy metal	13
Zinc Sulfate	heavy metal	12
Mercury	heavy metal	11
SB 203580	kinase inhibitor	11
PD 98059	kinase inhibitor	10
Hydralazine	medicinal	27
Valproic Acid	medicinal	26
Nicotine	medicinal	16
Simvastatin	medicinal	15
Nitroglycerin	medicinal	10
Pioglitazone	medicinal	10
Dicumarol	medicinal	9
Curcumin	natural product	45
1'-Acetoxychavicol acetate	natural product	32
Evodiamine	natural product	28
Dust	natural product	22
Plant Extracts	natural product	18
Acetylmuramyl-Alanyl-Isoglutamine	natural product	11
Cholesterol, HDL	natural product	10
Kojic acid	natural product	10
Catechin	natural product	10
Berberine	natural product	10

<u>Chemical name</u>	<u>Classification</u>	<u># gene interactions</u>
Phenylacetic acid	natural product	10
Zerumbone	natural product	9
tert-Butylhydroperoxide	oxidant	49
Hydrogen Peroxide	oxidant	28
Paraoxon	pesticide	18
Lipopolysaccharides	proinflammatory	111
Zymosan	proinflammatory	26
Dinoprostone	prostaglandin E2	10
Benzyloxycarbonylleucyl-leucyl-leucine aldehyde	proteasome inhibitor	12
Tretinoin	retinoid	162
Fenretinide	retinoid	22
Alitretinoin	retinoid	22
Isotretinoin	retinoid	15
Vitamin A	retinoid	14
4-Oxoretinoic acid	retinoid	10
Calcium	signalling	13
Pregna-4,17-diene-3,16-dione	steroid	18
Testosterone enanthate	steroid	18
Diosgenin	steroid	17
Metribolone	steroid	17
Mifepristone	steroid	15
Estradiol	steroid	89
Progesterone	steroid	46
Dexamethasone	steroid	26
Ethinyl Estradiol	steroid	11
Medroxyprogesterone 17-Acetate	steroid	11
Tetradecanoylphorbol Acetate	tumor promoter	37
Calcitriol	vitamin	25
Ascorbic Acid	vitamin	19
Folic Acid	vitamin	12