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DNA methylation alterations in response to pesticide exposure in vitro

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Abstract

Although pesticides are subject to extensive carcinogenicity testing before regulatory approval, pesticide exposure has repeatedly been associated with various cancers. This suggests that pesticides may cause cancer via non-mutagenicity mechanisms. The present study provides evidence to support the hypothesis that pesticide-induced cancer may be mediated in part by epigenetic mechanisms. We examined whether exposure to 7 commonly used pesticides (i.e., fonofos, parathion, terbufos, chlorpyrifos, diazinon, malathion, and phorate) induces DNA methylation alterations in vitro. We conducted genome-wide DNA methylation analyses on DNA samples obtained from the human hematopoietic K562 cell line exposed to ethanol (control) and several OPs using the Illumina Infinium HumanMethylation27 BeadChip. Bayesian-adjusted t-

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Authors' contributions

Zhang, Lin, Baccarelli, and Hou generated the study concept, study design, and prepared manuscript.

Wallace, Jafari, Xie and Soares performed the laboratory analyses.

Du, Lin, Kibbe performed bioinformatics analyses.

All authors read and approved the final manuscript.

tests were used to identify differentially methylated gene promoter CpG sites. In this report, we present our results on three pesticides (fonofos, parathion, and terbufos) that clustered together based on principle component analysis and hierarchical clustering. These three pesticides induced similar methylation changes in the promoter regions of 712 genes, while also exhibiting their own OP-specific methylation alterations. Functional analysis of methylation changes specific to each OP, or common to all three OPs, revealed that differential methylation was associated with numerous genes that are involved in carcinogenesis-related processes. Our results provide experimental evidence that pesticides may modify gene promoter DNA methylation levels, suggesting that epigenetic mechanisms may contribute to pesticide-induced carcinogenesis. Further studies in other cell types and human samples are required, as well as determining the impact of these methylation changes on gene expression.

Keywords

Pesticide exposure; DNA methylation alteration; Carcinogenesis

Introduction

Pesticides are widely used in our environment [Weichenthal et al. 2010]. Although pesticides sold in the US have passed the Environmental Protection Agency's (EPA) screening procedures for carcinogenicity (i.e., genotoxicity and mutagenicity tests [EPH 1997]), animal studies have shown that some of these pesticides are carcinogenic [Reuber 1981; Gupta et al. 2007; Wong and Matsumura 2007; Yu et al. 2008]. A number of pesticides have been repeatedly associated with various cancers in epidemiological investigations of farmers and pesticide manufacturing workers [Alavanja and Bonner 2005; Alavanja et al. 2007; Bassil et al. 2007; Koutros et al. 2010; Weichenthal et al. 2010; Waggoner et al. 2011]. The elevated cancer risk following exposure to pesticides indicates a gap in the current knowledge of pesticide carcinogenicity, and provides evidence that pesticides may cause cancer through alternative mechanisms, such as epigenetic changes [Skinner and Anway 2007; Alavanja 2009].

Methylation of 5'-CpG islands in gene promoter regions has consistently been found in malignant tissues and is hypothesized to be indicative of critical early changes in cancer development [Issa 2004]. DNA methylation alterations in gene promoters have also been found repeatedly following exposure to various environmental chemicals, including pesticides [Baccarelli and Bollati 2009]. Animal studies have shown that exposure to pesticides such as vinclozolin, methoxychlor, and dichlorvos, induce promoter DNA methylation alterations of multiple genes, including lysophospholipase, G protein-coupled receptor 33 (GPR33), potassium voltage-gated channel, Isk-related family, member 2 (KCNE2), and annexin A1 (ANXA1) [Hathaway et al. 1991; Anway and Skinner 2006; Guerrero-Bosagna et al. 2010]. Genomic DNA methylation content in blood leukocytes has been found to be inversely associated with plasma levels of pesticide residues in Arctic [Rusiecki et al. 2008] and Korean populations [Kim et al. 2010]. However, previous studies have been limited to the evaluation of very small sets of candidate methylation markers. Knowledge of the effects of pesticides on DNA methylation is limited, and DNA methylation alterations are not considered in carcinogenicity testing by the EPA or other agencies. The purpose of the present study was to conduct a genome-wide investigation to examine comprehensively whether exposure to three commonly used organophosphate pesticides (OPs) that have been associated with several cancers in human studies induce DNA methylation alterations in vitro.

Materials and Methods

Exposure of human K562 cells to three OPs

In vitro systems have previously been used to determine if exposure to chemicals alters DNA methylation [Watson et al. 2004; Bachman et al. 2006]. The human K562 cell line was derived from erythroblastic leukemia and can differentiate into recognizable progenitors of the erythrocytic, granulocytic, and monocytic series [Andersson et al. 1979; Lozzio et al. 1981; Baker et al. 2001]. Studies using K562 cells have demonstrated that DNA methylation can be altered by treatment with agents such as interleukin-6 and cadmium [Hodge et al. 2001; Huang et al. 2008] and were thus selected as a model for the present experiments. The K562 cell line was obtained from the American Type Culture Collection (ATCC, VA), and maintained in RPMI-1640 supplemented with 10% fetal calf serum, 100 µg/ml penicillin, and 100 U/ml streptomycin (Invitrogen, Carlsbad, CA). Pesticides were purchased from ChemService (West Chester, PA) and stock solutions were dissolved in ethanol. K562 cells were exposed to 7 individual pesticides (i.e., fonofos, parathion, terbufos, chlorpyrifos, diazinon, malathion, and phorate) at doses of 0.001, 0.01, and 0.1 µM, or ethanol (control) for different time periods (6, 12, 24, 48, and 72 hours). All the experiments were conducted in triplicate. Viability using triplicate samples was first assessed by the trypan blue exclusion assay using a hemocytometer for cell counting under an inverted microscope. Additionally, cell cytotoxicity was determined using the luciferase-based ToxiLight® (Lonza, Rockland, ME) assay system. Luminescence produced by luciferase is proportional to adenylate kinase release in the ToxiLight® assay and was measured in relative light units (RLUs) using a Fusion™ Universal Microplate Analyzer (Packard BioScience Company, Meriden, CT). These exposure dosages did not significantly affect cell viability (Supplementary Table 1; Supplementary Figure 1), and are similar to exposure levels experienced by pesticide applicators in real life [Fenske et al. 2002; Barr et al. 2005; Arcury et al. 2007; Arcury et al. 2009]. DNA was prepared with a Wizard Genomic DNA purification kit (Promega Corp, Madison, WI), quantified, and diluted into aliquots of 25 ng/µl for genome-wide DNA methylation analysis.

Genome-wide DNA methylation

Analysis of genome-wide DNA methylation was performed on DNA samples obtained from triplicate cell cultures exposed to each of the three pesticides (fonofos, parathion, and terbufos) at a dose of 0.1 µM for 12 hours using Illumina Infinium Human Methylation27 BeadChips, which contain 27,578 individual CpG sites covering the promoter regions of about 14,000 genes. The selection of the dose and time of exposure used in the current genome-wide investigation was based on our previous gene-specific methylation pilot experiment, in which K562 cells were exposed to several pesticides at different doses over different periods of time. Cells treated with pesticides at doses 0.1 µM for 12 hours or more demonstrated DNA methylation changes, and increasing doses did not generate significant differences in DNA methylation levels (unpublished data). Therefore, for genome-wide DNA methylation analysis, samples exposed at a dose of 0.1 µM for 12 hours were used. 500 ng of DNA was used to perform bisulfite conversion using the EZ-96 DNA Methylation Kit (Zymo Research, Orange, CA) following Illumina's protocol. Samples and controls for this study were dispersed in a 96-well plate with other samples to avoid inter-chip effects. Illumina BeadChips were scanned with an iScan and then analyzed using GenomeStudio software (Version 1.8.5, Illumina, Inc.). All experiments were conducted at the Genomics Core Facility of Northwestern University in accordance with the manufacturer's protocols. The 600 negative control probes and bisulfite conversion probes provided by Human Methylation27 were used to identify weak bead types and failed samples. Samples with < 75% detected loci were re-run. 5% replicates of 96 samples were interspersed with study samples in a 96-well plate using pseudo-participant IDs to mask

their origin from laboratory personnel. The concordance rates of the duplicate samples were 98 %. To further quantify and remove any inter-array effects, we developed our own quality control (QC) procedure by including commercially available known unmethylated (normal B-lymphocytes (Coriell: NA10923), Camden, NJ) and methylated (colon cancer cells (ATCC: HTB-38), Manassas, VA) control samples in each run [Du et al. 2010]. Unmethylated and methylated samples were mixed in ratios of 10:0, 9:1, 7.5:2.5, 5:5, and 0:10. For ten genes known to be hyper-methylated in cancer tissues [Bibikova et al. 2006], the resulting percentage of methylated cytosines over the sum of methylated and unmethylated cytosines (%mc) was plotted against the mixture ratio (Supplementary Figure 2). As illustrated, %mc varied directly with the mixture ratio on duplicate runs (mean $r = 0.99$).

Bioinformatics/biostatistics analysis

The methylation microarray data were processed using the Bioconductor lumi package [Du et al. 2008]. The GenomeStudio output data first went through a quality assurance (QA)/QC step. For the samples passing QA/QC, we performed a color balance adjustment of methylated and unmethylated probe intensities between two color channels using the smooth quantile normalization method. The methylated and unmethylated probe intensities were then normalized using the Simple Scaling Normalization (SSN) method. Methylation M-value ($\log_2$ ratio of methylated and unmethylated probes) was used to detect the differential methylation of each CpG-site, whereas the Beta-value (rescaled between 0 and 1) was used for visualization [Du et al. 2010]. Identification of changes in methylation data (exposed relative to control) is similar to expression microarray data. We applied routines implemented in the LIMMA package [Smyth 2004] to fit linear models to the normalized M-values. To ensure the selected CpG-sites had both high statistical significance and strong biological effects, we identified differentially methylated CpG-sites based on the following criteria: False Discovery Rate (FDR)-adjusted P -value (i.e., q -value) < 0.05 and an absolute DNA methylation fold-change > 2.00 (M-value is defined in the $\log_2$ scale, the fold-change is defined as $2^{M_t - M_c}$, where M_t and M_c be the average methylation levels of pesticide-treated and control groups). All analyses were based on the mean of biological triplicates. CpG-sites with significantly different methylation levels were then mapped to the closest downstream genes. We identified CpG sites with significant methylation changes in response to exposure to each individual pesticide that we have examined. In this report, we present our results on three pesticides (fonofos, parathion, and terbufos) that had comparable changes in methylation for 712 genes, while also exhibiting their own OP-specific methylation alterations (GEO accession number: GSE38646). Gene ontology analysis of genes specifically responsive to each OP, or common to all three OPs, revealed that differential methylation was found nearby various genes that are involved in carcinogenesis-related processes using the Bioconductor GeneAnswers package [Feng et al. 2010].

Pyrosequencing verification

Four genes were pyrosequenced to validate the genome-wide DNA methylation data: aristalesslike homeobox 4 (*ALX4*), histone deacetylase 5 (*HDAC5*), O-6-methylguanine-DNA methyltransferase (*MGMT*), and telomerase reverse transcriptase (*TERT*). The selection of these genes was based on statistical significance (i.e. differentially methylated by all three pesticides, showing methylation fold-change > 2.00 and q -value < 0.01) and cancer-related biological functions. PCRs were carried out using the Hotstart Taq polymerase kit (Qiagen, Valencia, CA) in a total volume of 25 μ L and with 50 pm of forward primer and reverse primer. For each PCR, 50 ng of the bisulfite-converted DNA was used as a template. Bisulfite modification of genomic DNA was performed using an EZ DNA Methylation Gold kit according to the manufacturer's instructions (Zymo Research, Orange, CA). Pyrosequencing was performed using the PyroMark MD Pyrosequencing System (Biotage,

Charlottesville, VA) as described previously [Xie et al. 2009]. Methylation quantification was performed using the manufacturer's software. Primer sequences are shown in Supplementary Table 2.

Results

An overview of the sample relations based on the principal component (PCA) (Figure 1) and hierarchical cluster analysis (Supplementary Figure 3) of genome-wide DNA methylation profiles, as well as a heatmap of all differentially methylated genes (Figure 2A), showed distinct methylation patterns in fonofos, parathion, and terbufos-treated cells compared with other OP-treated (i.e. chlorpyrifos, diazinon, malathion, and phorate) or control cells (unpublished data). Here, we present our results on the three pesticides (fonofos, parathion, and terbufos) that clustered together based on principle component analysis and hierarchical clustering. We identified 1759, 1746, and 1580 CpG sites with significant methylation changes in response to exposure to fonofos, parathion, and terbufos, respectively. In total 712 promoter sites had comparable levels of methylation changes for all three pesticides, including 625 hypermethylated (Figure 2B) and 87 hypomethylated genes (Figure 2C). Functional analysis by GO terms showed that some of these genes are implicated in cancer development or in related biological pathways, while some are functionally unknown (Table I).

We observed 712 promoters with similar DNA methylation changes for all three OPs. Some of the genes associated with these promoters have been implicated in carcinogenesis. For example, tumor protein p53 inducible protein 11 (*TP53I11*) exhibited increased DNA methylation for all three OPs (4.0-fold for fonofos, q-value < 0.001; 4.7-fold for parathion, q-value < 0.001; 3.1-fold for terbufos, q-value < 0.001; respectively). Other genes with increased DNA methylation in all three OP-exposed cell lines include *ALX4* (11.9-fold for fonofos, q-value < 0.001; 13.7-fold for parathion, q-value < 0.001; 16.3-fold for terbufos, q-value < 0.001; respectively), pancreatic and duodenal homeobox 1 (*PDX1*) (8.8-fold for fonofos, q-value < 0.001; 9.1-fold for parathion, q-value < 0.001; 3.4-fold for terbufos, q-value = 0.01; respectively), and WNT1 inducible signaling pathway protein 3 (*WISP3*) (3.2-fold for fonofos, q-value = 0.007; 3.5-fold for parathion, q-value = 0.003; 3.8-fold for terbufos, q-value = 0.002; respectively) (Table I).

Some genes with biological functions related to cancer etiology, such as immune response, DNA repair, and telomere length maintenance, were also identified as having altered promoter methylation following exposure to all three of the OPs including: (1) growth arrest and DNA-damage-inducible gamma (*GADD45G*) (25.2-fold for fonofos, q-value < 0.001; 23.1-fold for parathion, q-value < 0.001; 31.2-fold for terbufos, q-value < 0.001; respectively); (2) *MGMT* (9.1-fold for fonofos, q-value < 0.001; 6.8-fold for parathion, q-value = 0.001; 13.7-fold for terbufos, q-value < 0.001; respectively); (3) *TERT* (7.0-fold for fonofos, q-value = 0.01; 9.8-fold for parathion, q-value = 0.003; 10.8-fold for terbufos, q-value = 0.002; respectively); and (4) interleukin-1 receptor (*IL1RI*) (-2.2-fold for fonofos, q-value = 0.001; -2.1-fold for parathion, q-value = 0.002; -2.2-fold for terbufos, q-value = 0.001; respectively). Aberrant promoter methylation of proliferation-related genes was found including: cyclin-dependent kinase 10 (*CDK10*) (7.0-fold for fonofos, q-value < 0.001; 5.5-fold for parathion, q-value < 0.001; 7.2-fold for terbufos, q-value < 0.001; respectively); protein tyrosine phosphatase, receptor type, K (*PTPRK*) (16.9-fold for fonofos, q-value < 0.001; 14.5-fold for parathion, q-value < 0.001; 12.0-fold for terbufos, q-value < 0.001; respectively); and protein tyrosine phosphatase, receptor type, G (*PTPRG*) (4.1-fold for fonofos, q-value < 0.001; 3.9-fold for parathion, q-value < 0.001; 4.3-fold for terbufos, q-value < 0.001; respectively) (Table I).

Several promoters associated with genes of unknown biological functions exhibited very large changes in methylation levels. For example, the promoter of DAZ interacting protein 1 (*DZIP1*), a mammalian protein with unknown biological function, showed large fold changes (34.4-fold for fonofos, q-value < 0.001; 39.5-fold for parathion, q-value < 0.001; 22.9-fold for terbufos, q-value < 0.001; respectively). A series of genes producing hypothetical proteins showed large increases in promoter DNA methylation, such as hypothetical protein LOC123904 (*UNQ2446*) (13.2-fold increase for fonofos, q-value < 0.001; 13.1-fold for parathion, q-value < 0.001; 13.9-fold for terbufos, q-value < 0.001; respectively), LOC255101 (*DKFZp434O0527*) (11.4-fold increase for fonofos, q-value < 0.001; 17.1-fold increase for parathion, q-value < 0.001; 11.2-fold for terbufos, q-value < 0.001; respectively), and LOC55264 (*C21orf77*) (9.5-fold increase for fonofos, q-value < 0.001; 10.0-fold for parathion, q-value < 0.001; 10.7-fold for terbufos, q-value < 0.001; respectively) (Table I).

Pyrosequencing results for three selected promoters were highly correlated with the genome-wide findings, including *ALX4* ($R^2 = 0.86$), *MGMT* ($R^2 = 0.81$) and *TERT* ($R^2 = 0.91$) (Supplementary Figure 4).

Discussion

Distinct genome-wide DNA methylation patterns were observed in cell cultures exposed to three commonly used OPs (fonofos, parathion, and terbufos) relative to controls. In fonofos-, parathion-, and terbufos-treated cells, we identified 1759, 1746, and 1580 CpG sites with significant methylation changes in 1551, 1482, and 1357 genes, respectively. Of these, 712 sites had similar methylation changes for all three OPs (Figure 2). Gene ontology analysis demonstrated that a number of the genes associated with these loci are directly implicated in carcinogenesis, with functions related to cancer etiology, while some with large fold changes were associated with genes having unknown functions (Table I). The three OPs in our study are associated with various cancers (Table II), including colorectal [Lee et al. 2007], breast [Cabello et al. 2001; Cabello et al. 2003; Calaf et al. 2009], lung [Dennis et al. 2010], and skin cancers [Bonner et al. 2010], in experimental and human studies. Therefore, the overlap in methylation changes observed here for the OPs may provide evidence of commonalities in the biological pathways contributing to carcinogenesis.

Pesticides may affect DNA methylation through several cellular processes, including oxidative stress/reactive oxygen species generation [Fratelli et al. 2005; Yu et al. 2008], changes in DNA methyltransferase activity [Shepherd et al. 2006], and immunotoxicity [Daniel et al. 2001; Galloway and Handy 2003]. In experimental and human studies, several OPs have been shown to induce oxidative stress [Mena et al. 2009; Soltaninejad and Abdollahi 2009]. DNA methylation reflects cumulative oxidative stress, and ROS production has recently been shown to alter the expression of genes belonging to DNA methylation machinery [Fratelli et al. 2005]. We examined DNA machinery-related genes in detail from our profiles, and found that *DNMT3A* methylation was increased (2.2-fold for fonofos, q-value = 0.01; 2.4-fold for parathion, q-value = 0.003), while *DNMT3B* was decreased (-2.2-fold for parathion, q-value = 0.002; -2.8-fold for terbufos, q-value = 0.003), suggesting that pesticides may perturb DNA methylation via altered expression of DNA methylation machinery. Some pesticides have immunotoxic effects [Veraldi et al. 2006] and are associated with elevated cytokine levels [Phillips 2000]. It has been reported that elevated plasma cytokine levels were associated with hypermethylation of tumor-suppressor genes in peripheral lymphocytes [Daniel et al. 2001]. These factors may independently or coordinately affect DNA methylation. Further research is needed to better precisely define the mechanisms leading to pesticide-induced DNA methylation changes.

Our findings should be interpreted with caution, as there were a few limitations to this study. For example, we used K562 cells, a chronic myelogenous leukemia cell line that resembles multi-potent hematopoietic cells. Cancer cell lines may be pre-disposed to effects on DNA methylation. However, published studies using K562 cells have demonstrated that DNA methylation can be altered by treatment with agents such as interleukin-6 and cadmium, suggesting K562 cell DNA methylation is inducible by chemical exposures, including pesticides. Furthermore, all changes were relative to K562 controls, and the work clearly shows that the three different OPs exhibit a substantial overlap in altered methylation sites. Additional studies should determine if similar alterations in DNA methylation patterns arise in other cell types after exposure to these pesticides. Another limitation of our investigation is that we did not determine whether the observed methylation alterations lead to gene expression changes at the transcriptional or protein levels. Future studies should clarify this, in addition to considering other epigenetic markers, such as histone modification and microRNAs.

Our results provide direct experimental evidence that three pesticides that are associated with increased cancer risk in humans cause changes in promoter CpG methylation in numerous genes. These data, coupled with human epidemiology evidence linking pesticides with cancers and recent evidence indicating DNA methylation alterations as a hallmark of cancer, support the notion that pesticide exposure may lead to cancer in part via inducing alterations in DNA methylation. Further studies in different cell lines and in vivo are warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

Distinct methylation patterns of the fonofos, parathion, and terbufos-treated cells. Overview of the sample relations based on PCA plot of genome-wide DNA methylation profiles clearly showed distinct methylation patterns of fonofos, parathion, and terbufos-treated cells (green) in comparison with other OPs-treated or control cells (black). Para: parathion; Terb: terbufos; Fono: fonofos; Chlo: chlorpyrifos; Dich: Dichlorvos; Diaz: diazinon; Phor: phorate; Ctrl: control. 0, 1, and 2 represent triplicate runs for each sample. 0, 1, 2, 3, 4 and 5 represent six runs for control.

All differentially methylated genes (panel A) and common genes with similar DNA methylation changes among three OPs (panel B, C). Panel A. DNA methylation heatmap of all differentially methylated genes in fonofos, parathion, and terbufos-treated cells compared with other OPs-treated or control cells. The heatmap color corresponds to the Beta-value of the measured CpG-sites. The Beta-value is in the range of 0 (shown in green) and 1 (shown in red) with 0 representing purely unmethylated and 1 representing purely methylated. The color bar above the heatmap represents the sample types, in which the red color represents three concordant pesticides including fonofos, parathion, and terbufos; black corresponds to the control samples and green represents all other pesticide-treated samples. Panel B and C. Venn diagram showing genes with comparable levels of methylation changes in all three OPs, including 625 hypermethylated genes (B) and 87 hypomethylated genes (C).

Table I

Selected genes related to cancer based on GO analysis

Gene Symbol *	Entrez ID	Fonofos		Parathion		Terbufos		Function
		Fold	q-value	Fold	q-value	Fold	q-value	
DZIP1	22873	34.4	<0.001	39.5	<0.001	22.9	<0.001	Cell differentiation
GADD45G	10912	25.2	<0.001	23.1	<0.001	31.2	<0.001	Cell differentiation
PTPRK	5796	16.9	<0.001	14.5	<0.001	12.0	<0.001	Regulation of cell proliferation
UNQ2446	123904	13.2	<0.001	13.1	<0.001	13.9	<0.001	Anchored to membrane
ALX4	60529	11.9	<0.001	13.7	<0.001	16.3	<0.001	Regulation of apoptosis
DKFZp434O0527	255101	11.4	<0.001	17.1	<0.001	11.2	<0.001	Integral to membrane
C21orf77	55264	9.5	<0.001	10.0	<0.001	10.7	<0.001	N/A
MGMT	4255	9.1	<0.001	6.8	0.001	13.7	<0.001	DNA dealkylation involved in DNA repair
PDX1	3651	8.8	<0.001	9.1	<0.001	3.4	0.01	Cell differentiation
PAX7	5081	8.1	<0.001	10.1	<0.001	9.8	<0.001	Cell differentiation
TERT	7015	7.0	0.01	9.8	0.003	10.8	0.002	Telomere maintenance
CDK10	8558	7.0	<0.001	5.5	<0.001	7.2	<0.001	Regulation of cell proliferation
PTPRG	5793	4.1	<0.001	3.9	<0.001	4.3	<0.001	Regulation of cell proliferation
TP53I11	9537	4.0	<0.001	4.7	<0.001	3.1	<0.001	Negative regulation of cell proliferation
TNFRSF11B	4982	3.8	0.003	3.6	0.004	4.2	0.001	Response to hormone stimulus
TNFRSF25	8718	2.8	<0.001	4.1	<0.001	2.8	<0.001	Positive regulation of apoptosis
HIST4H4	121504	2.8	<0.001	3.1	<0.001	2.8	<0.001	Chromatin modification; histone deacetylation
HDAC5	10014	2.6	<0.001	3.0	<0.001	2.0	0.01	Regulation of cell proliferation
WISP3	8838	3.2	0.007	3.5	0.003	3.8	0.002	Cell-cell signaling
IL1R1	3554	-2.2	0.001	-2.1	0.002	-2.2	0.001	Cell surface receptor linked signaling pathway

DZIP1: DAZ interacting protein 1; GADD45G: growth arrest and DNA-damage-inducible, gamma; PTPRK: protein tyrosine phosphatase, receptor type, K; UNQ2446: hypothetical protein LOC123904; ALX4: aristaless-like homeobox 4; DKFZp434O0527: hypothetical protein LOC25510; C21orf77: hypothetical protein LOC55264; MGMT: O-6-methylguanine-DNA methyltransferase; PDX1: pancreatic and duodenal homeobox 1; PAX7: paired box gene 7 isoform 1; TERT: telomerase reverse transcriptase isoform 3; CDK10: cyclin-dependent kinase 10; PTPRG: protein tyrosine phosphatase, receptor type, G; TP53I11: tumor protein p53 inducible protein 11; TNFRSF11B: tumor necrosis factor receptor superfamily, member 11b; TNFRSF25: tumor necrosis factor receptor superfamily, member 25; HIST4H4: histone H4; HDAC5: histone deacetylase 5; WISP3: WNT1 inducible signaling pathway protein 3; IL1R1: interleukin 1 receptor, type I

* OP-specific genes and common genes in 3 OPs were sorted by absolute fold change in fonofos-treated cells

Table II

Profile of fonofos, parathion and terbufos

Pesticide	*Toxicity classification	**EPA group	Chemical structure	Association with Cancers	Species
fonofos	Toxicity Category I	Group E		Colon cancer (Lee et al. 2007)	Human
				Leukemia (Lee et al. 2007)	Human
				PC (Mahajan et al. 2006)	Human
				NHL (De Roos et al. 2003)	Human
parathion	Toxicity Category I	Group C		Breast cancer (Cabello et al. 2003; Calaf et al. 2009)	<i>In vitro</i>
				Breast cancer (Cabello et al. 2001)	Rat
				Liver cancer (Coral et al. 2009; Pasquini et al. 1994)	Rat
				Melanoma (Dennis et al. 2010)	Human
terbufos	Toxicity Category I	Group E		PC (Bonner et al. 2010; Mahajan et al. 2006)	Human
				Lung cancer (Bonner et al. 2010)	Human
				Leukemia (Bonner et al. 2010)	Human
				NHL (Bonner et al. 2010)	Human

* Toxicity classification: **Category I**, most toxic, **Category II**, moderately toxic; **Category III**, slightly toxic, **Category IV**, practically nontoxic.

** EPA carcinogenicity classification: **A**, known to cause cancer in humans; **B**, probable human carcinogen; **C**, possible human carcinogen; **D**, not classifiable as to human carcinogenicity; and **E**, probably not carcinogenic