Environmental Research 182 (2020) 109131

Contents lists available at ScienceDirect

Environmental Research

journal homepage: www.elsevier.com/locate/envres

Malathion induced cancer-linked gene expression in human lymphocytes

Ramakrishnan Anjitha[a], Anet Antony[a], Olakkaran Shilpa[a], Kizhakke P. Anupama[a], Shanthala Mallikarjunaiah[b], Hunasanahally P. Gurushankara[a,*]

[a] Department of Zoology, School of Biological Sciences, Central University of Kerala, Periya, 671 320, Kasaragod, Kerala, India
[b] Center for Applied Genetics, Department of Studies in Zoology, Bangalore University, Jnanabharathi, Bengaluru, 560 056, Karnataka, India

ARTICLE INFO

Keywords:
Malathion
Pesticides
Cancer
Gene expression
Microarray

ABSTRACT

Background: Malathion is the most widely used organophosphate pesticide in agriculture. Increasing cancer incidence in agricultural workers and their children links to the exposure of malathion. Identification of genes involved in the process of carcinogenesis is essential for exploring the role of malathion. The alteration in gene expression by malathion in human lymphocytes has not been explored yet, although hematological malignancies are rampant in humans.

Objective: This study investigates the malathion induced expression of cancer associated genes in human lymphocytes.

Methods: Human lymphocyte viability and colony-forming ability were analyzed in malathion treated and control groups. Gene expression profile in control and malathion treated human lymphocytes were performed using a microarray platform. The genes which have significant functions and those involved in different pathways were analyzed using the DAVID database. Differential gene expression upon malathion exposure was validated by quantitative real-time (qRT)-PCR.

Results: Malathion caused a concentration-dependent reduction in human lymphocyte viability. At low concentration (50 μg/mL) of malathion treatment, human lymphocytes were viable indicating that low concentration of malathion is not cytotoxic and induces the colony formation. Total of 659 genes (15%) were up regulated and 3729 genes (85%) were down regulated in malathion treated human lymphocytes. About 57 cancer associated genes related to the growth and differentiation of B and T cells, immunoglobulin production, haematopoiesis, tumor suppression, oncogenes and signal transduction pathways like MAPK and RAS were induced by malathion.

Conclusion: This study evidences the carcinogenic nature of malathion. Low concentration of this pesticide is not cytotoxic and induces differentially regulated genes in human lymphocytes, which are involved in the initiation, progression, and pathogenesis of cancer.

1. Introduction

Malathion (Diethyl 2-[(dimethoxyphosphorothioyl)sulfanyl] butanedioate) is a non-systemic broad-spectrum organophosphate pesticide used for controlling insect pests of agricultural crops, gardens, house hold products, ectoparasites on animals, fumigation, veterinary practices and in public health pest-eradication programmes (ATSDR, 2003). Malathion residues have been frequently detected in the agroecosystem sediment (2.62–129 μg/kg); water (0.699–298 μg/L); ground water near industrial area (0.855–16.24 μg/L); rain water (0.900–1.500 μg/L); river surface water (2.618 μg/L); unfiltered water (0.1–3.3 μg/L); drinking water pipes (0.2–1.0 mg/g); urine (1.3–4.1 μg/L); fruits and vegetables (0.04 ng/g) (Rao and Pillala, 2001; Varca, 2012; Agarwal et al., 2015; Harper et al., 2017; IARC , 2017). Malathion exposure can cause acute and chronic toxicity, disturbance in metabolism, induce oxidative stress, decrease in AChE activity, hepatotoxicity, neurotoxicity, immunotoxicity, cytotoxicity and genotoxicity in target and non-target species including humans (Windham et al., 1998; Gurushankara et al., 2007a; 2007b; Muniz et al., 2008; Kalender et al., 2010; Moore et al., 2010; Nain et al., 2011; Akbel et al., 2018; Selmi et al., 2018).

Cancer is the most common malignant disease, causing major morbidity and mortality in the world. It is a genetic disease, i.e., the initiation of cancer occurs at the molecular level due to alterations in genes, proteins and the signal transduction pathways that control the way our cells function, specifically how they grow and divide (Hanahan and Weinberg, 2000). The causative factors for carcinogenesis remain

* Corresponding author.
E-mail address: hpgurushankara@gmail.com (H.P. Gurushankara).

https://doi.org/10.1016/j.envres.2020.109131
Received 5 June 2019; Received in revised form 8 January 2020; Accepted 9 January 2020
Available online 10 January 2020
0013-9351/

an enigma. Pesticides are considered as carcinogens and appear to be involved in the etiology of cancer (Mitra et al., 2012; Goodson et al., 2015). But, there is no report to propose how pesticides may influence gene expression in carcinogenesis. Identification of genes involved in the process of carcinogenesis upon pesticide exposure is essential for exploring the role of environmental agent(s) in cancer development. Studies have substantiated that malathion induces DNA and chromosomal damages in humans (Moore et al., 2010; Navarrete-Meneses et al., 2018). Increased cancer (particularly leukemia and lymphoma) incidence in agriculture workers and their children was linked to malathion exposure (Cabello et al., 2001; Bonner et al., 2007; Lerro et al., 2015). Malathion could be a carcinogen as its exposure was found to elevate the risk of cancer (Reuber, 1985; Cabello et al., 2001). International Agency for Research on Cancer (IARC) of the World Health Organization (WHO) has classified malathion as 'probably carcinogenic to humans' (Group 2A) (IARC , 2017). However, the alteration in the gene expression by malathion has not been explored so far, although hematological malignancies are prevalent in humans. In order to understand the genes involved in carcinogenesis, we have employed the gold standard *in vitro* model system, human lymphocytes. The profile of malathion induced cancer related gene expression in human lymphocytes were made using microarray platform and differential gene expressions were confirmed by quantitative real time (qRT)-PCR.

2. Methods

2.1. Isolation of human lymphocytes and culture

Human peripheral blood (5 mL) was collected by venipuncture from non-smoker, non- drinker and medication-free healthy male and female volunteers of age between 20 and 23 years (21.11 ± 0.745) in vacutainer blood tubes containing sodium heparin (Cat. No. 367878, Becton-Dickinson, India Pvt. Ltd). The investigation was conducted with the approval of the Central University of Kerala, Institutional Human Ethical Committee (CUK/IHEC/2017–011) and volunteer blood was drawn after obtaining the informed consent. Human lymphocytes were isolated by Ficoll-Paque density gradient method. Briefly, 5 mL of blood was diluted with 5 mL of sterile phosphate buffered saline (PBS), pH 7.4 (1:1 ratio). Total 10 mL of diluted blood was layered on the 5 mL of Ficoll-Paque containing centrifuge tube and was centrifuged at 400 g for 20 min at room temperature (20 °C) to obtain a buffy layer (containing lymphocytes) between the plasma and Ficoll-Paque layers. The obtained buffy coat was washed twice with sterile PBS at 100 g for 10 min each and then washed with RPMI-1640 culture media by centrifugation. Human lymphocyte density was determined by Trypan blue exclusion test and was found to be about 95%. Human lymphocytes (1×10^4) were cultured in RPMI-1640 medium supplemented with 10% fetal bovine serum (FBS), 1.5% phytohemagglutinin (PHA) and 1% of penicillin-streptomycin solution (antibiotic and antimycotic). Lymphocytes were cultured in a humidified incubator with 5% CO_2 at 37 °C in 15 mL T25 polystyrene culture flasks for 48 h. The viability of cultured human lymphocytes was checked before starting the experiment with the Trypan blue exclusion technique and was found to be greater than 85%.

2.2. Malathion stock preparation, toxicity analysis and selection of concentrations for assays

Malathion (μg/mL) stock solution was prepared in 1% dimethyl sulfoxide (DMSO). Human lymphocytes were treated with different concentrations of malathion by adding the appropriate volume of stock solution to the fresh RPMI-1640 growth media supplemented with 10% FBS, 1.5% PHA and 1% penicillin-streptomycin solution. IC_{50} of malathion for human lymphocytes was determined; i.e., the concentration at which 50% of lymphocytes failed to be viable/proliferate. To determine the 50% of an inhibitory concentration of malathion, 1×10^4 lymphocytes were seeded in 100 μL of RPMI-1640 medium containing suitable supplements in each well of 96-well microtiter plate in triplicate. The plate was incubated for a period of 24 h in a humidified incubator with 5% CO_2 at 37 °C. Human lymphocytes grown to 80–100% confluence were exposed to various concentrations (ranging from 0 to 2000 μg/mL) of malathion. Human lymphocytes treated with 1% DMSO without malathion was considered as vehicle control and lymphocytes untreated with 1% DMSO or malathion was considered as a control group. The lymphocyte viability determined using the Trypan blue exclusion test, indicated that there was no difference in lymphocyte count between the 1% DMSO treated and untreated control groups. Hereafter, 1% DMSO vehicle control was used as a control group which did not affect the lymphocyte viability under this study. The control and various concentrations of malathion treated lymphocytes were incubated for 24 h in a humidified incubator with 5% CO_2 at 37 °C. After the incubation, lymphocyte viability was evaluated using the XTT (2,3-bis-(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxanilide) assay. In brief, after the 24 h incubation, 50 μL XTT solution (freshly prepared activated XTT) per well was added. The 96-well plate was again incubated for a period of 2 h in a humidified CO_2 incubator with 5% CO_2 at 37 °C. In living cells, XTT is metabolically reduced to produce a colorimetric, water-soluble formazan product, which is in orange color. The appearance of orange color in the sample containing wells was noted and the plate was gently shaken several times to evenly distribute the orange color in each well. The absorbance was measured at 450 nm using a multimode plate reader (EnSpire™ multimode plate reader, PerkinElmer, Inc.). The amount of color produced is directly proportional to the number of viable cells. Lymphocyte viability was determined by comparing the absorbance of the wells containing the malathion treated groups with those of the control and blank. The assay was conducted in triplicate and was repeated thrice. The data of each malathion treated and control groups were used to determine the IC_{50} by Probit method (Finney, 1971). Calculated IC_{50} for malathion was 243.56 μg/mL. Hence, the sub lethal concentrations (i.e. 50, 100, and 150 μg/mL) of malathion were fixed for analyzing the human lymphocytes viability, colony formation assay, and qRT-PCR analysis. The low concentration of malathion (50 μg/mL) was selected for microarray analysis, as the human lymphocytes were found to be viable and proliferating (Fig. 1).

2.3. Colony forming assay

The colony forming potential of human lymphocytes was analyzed by the method of Crowley and Waterhouse (2016). Briefly, human lymphocytes were cultured in RPMI-1640 medium containing suitable

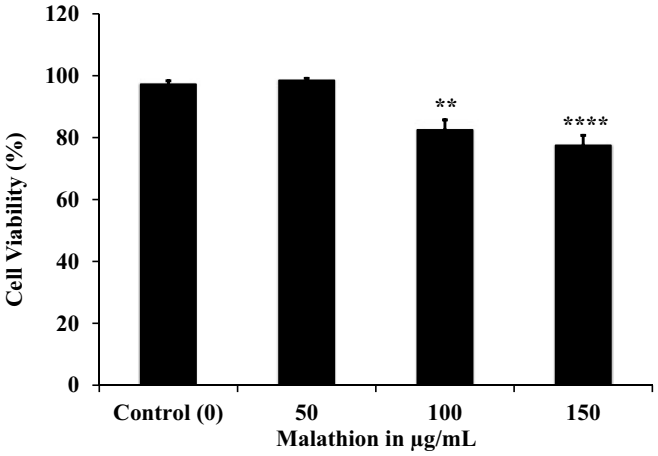

Fig. 1. Effect of malathion on human lymphocytes viability. Values without the symbol (*) is not significant compared to control at $p > 0.05$; significant compared to control at $^{**}p < 0.01$; $^{****}p < 0.0001$ level.

supplements in a humidified incubator with 5% CO_2 at 37 °C in 15 mL T25 polystyrene culture flasks for 48 h. The colony forming assay plate was prepared as follows: The bottom layer for the assay was prepared by mixing noble agar (1.3%) with the bottom and top layer media (2X RPMI-1640 medium supplemented with 10% FBS, 1.5% PHA and 1% of penicillin-streptomycin) at a ratio of 55:45 and immediately poured 2.5 mL into 6 cm dishes. These bottom layered assay plates were incubated overnight in a humidified incubator with 5% CO_2 at 37 °C to solidify. Human lymphocytes (1×10^4/dish) in triplicate were treated with different concentrations of malathion (50, 100 and 150 μg/mL) along with control. The control and treated lymphocytes were cultured for 24 h in a humidified incubator with 5% CO_2 at 37 °C. The cell layer of the assay was prepared by resuspending the 1 ml of respective treated and control 1×10^4 lymphocytes with the 9 mL of cell layer media/agar mix of the assay. The cell layer media/agar mix was prepared by mixing the 2X and 1X RPMI-1640 medium containing suitable supplements with noble agar (1.3%) at a ratio of 80:20. Immediately, the respective 5 mL of cell/media/agar mixture was added on the top of the solid bottom layer of agar and incubated at room temperature for 30 min to solidify. The top layer of the assay was prepared by mixing noble agar (1.3%) with the bottom and top layer media at a ratio of 55:45 and 2.5 mL of the top layer media was immediately poured on top of the solidified cell layer. The control and different sublethal concentrations of malathion treated assay dishes were cultured in an incubator with 5% CO_2 at 37 °C for 2–3 weeks until colonies were visible. The colonies formed were then counted using the dissection microscope. The colony having more than 50 cells were- considered for the analysis.

2.4. RNA extraction for microarray analysis

RNA was extracted from the samples by TRIzol reagent method. Control and malathion treated respective cultured human lymphocytes were harvested in pre-chilled 1X PBS in centrifuge tubes. The tubes were centrifuged at 2000 rpm for 15 min at 4 °C. The supernatant media was discarded carefully and the pellet was collected. The pellet was diluted by adding 4–5 volume of sterile and pre-chilled 1X PBS solution. The tubes were gently tapped for 2 min (care has been taken to avoid rupture of the cell membrane by vigorous shaking). The tubes were centrifuged at 2000 rpm for 15 min at 4 °C. The supernatant was discarded carefully and the pellet was collected. To the pellet 500 μL TRIzol was added and vortexed for 10 min for getting the homogenate. The tubes were centrifuged at 8000 rpm for 4 min to check for proper cell lysis. If the pellet was observed at the bottom, then vortexed again for 10 min 100 μL of chloroform was added to the sample tubes, securely capped and mixed by inversion for 15 s. The tubes were centrifuged at 13200 rpm for 15 min at 4 °C. After centrifugation, the mixture was separated into a lower red phenol-chloroform phase, interphase, and a colorless upper aqueous phase. The aqueous phase was transferred to a fresh tube. 250 μL of isopropyl alcohol was added to the aqueous phase to precipitate the RNA. Samples were incubated at room temperature for 10 min and centrifuged at 13200 rpm for 15 min at 4 °C. The RNA was precipitated at the side and bottom of the tube forming a gel-like pellet. The supernatant was discarded. The RNA pellet was washed twice with 0.5 mL 70% ethanol. The sample was mixed and centrifuged at 13200 rpm for 5 min at 4 °C. The RNA pellet was vacuum dried for 3 min at 30 °C. The RNA was dissolved in nuclease free water and stored at −80 °C. The stored RNA was used for microarray analysis.

2.5. RNA analysis

Total RNA and complementary RNA (cRNA) was purified using Qiagen's RNeasy Minikit (Cat#74106). RNA quality and purity were analyzed using the Nanodrop ND-1000, and RNA integrity was analyzed using the Agilent 2100 Bioanalyzer. Samples achieved A260/A280 absorbance ratios of purified RNA exceeding 2.0, the 28S/18S rRNA ratios were equal to or exceeded 1.5, and the RNA integrity number (RIN) exceeded 8.0.

2.6. Acquiring microarray data

The samples were labeled using the Agilent's Quick-Amp labeling kit (p/n:5190–0442). T7 promoter based-linear amplification method was used to generate labeled complementary RNA (One-color microarray-based gene expression analysis). The hybridization was done using Agilent's *in situ* hybridization kit (5190–6420). Gene expression was assessed on the Human 8 × 60K GXP AMADID:039494 commercially available *in situ* oligonucleotide arrays (A-GEOD-19775-Agilent-062647 INP 039494 Human GE v2 8 × 60K 039494) according to standard expression analysis protocols.

2.7. Analysis of microarray data

The normalization was made between the control and the malathion treated sample data using GeneSpring GX Software. Percentile shift normalization was employed to adjust the locations of all the spot intensities in the array. The number of differentially regulated genes consisting of up regulated genes (fold value ≥ 1) and down regulated genes (fold value ≤ -1) in the treated sample were found out. The color range was from green to yellow to red. The control was represented by a yellow color, the up regulated genes by red and the down regulated genes by green color. The fold change was calculated as log base 2. The genes which are present in significant functions and pathway were analyzed using the DAVID database (http://david.abcc.ncifcrf.gov/home.jsp).

2.8. Validation of malathion induced differential gene expression by quantitative RT-PCR

To validate the malathion induced cancer associated gene expression in human lymphocytes, *LPCAT4, MICA, BMI1, MAP2K3* and *HLA-E* genes were selected on the basis of their highest fold changes recorded in microarray analysis. *ACTB* gene was used for normalization. The forward and reverse primers of these genes (Table 1) were designed and obtained from Integrated DNA Technologies (IDT) USA. Briefly, lymphocytes were cultured in RPMI-1640 medium containing suitable supplements in a humidified incubator with 5% CO_2 at 37 °C in 15 mL T25 polystyrene culture flasks for 48 h. The cultured 1×10^4 lymphocytes/group in triplicate were treated with different sublethal concentrations (50, 100 and 150 μg/mL) of malathion along with control and were cultured for 24 h. The malathion treated and control lymphocytes were harvested and RNA was isolated from each sample by TRIzol reagent method. RNA was quantified and normalized to 1 μg. 20 μL of cDNA was synthesized using a high capacity Verso cDNA synthesis kit (Thermo Fisher Scientific). The relative quantification of gene expression was analyzed by LightCycler® 480 Instrument II - Roche Life Science using SYBR Green qPCR master mix. Each sample was run in triplicate for each gene. The gene expression levels of samples were then analyzed by calculating ΔCT and ΔΔCT values: ΔCT

Table 1
List of genes and their primers used for validation of gene expression by qRT-PCR.

Gene	Forward primer	Reverse primer
LPCAT4	GCTACAGCTCTCTGATCCTCAG	GACGAGTTAGCTCTTCCAGGCT
MICA	CCACCAGGATTTGCCAAGGAGA	CTGCCAATGACTCTGAAGCACC
BMI1	GGTACTTCATTGATGCCACAACC	CTGGTCTTGTGAACTTGGACATC
MAP2K3	CTTGGTGACCATCTCAGAACTGG	CTTCTGCTCCTGTGAGTTCACG
HLA-E	CGGCTACTACAATCAGAGCGAG	AATCCTTGCCGTCGTAGGCGAA
ACTB	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT

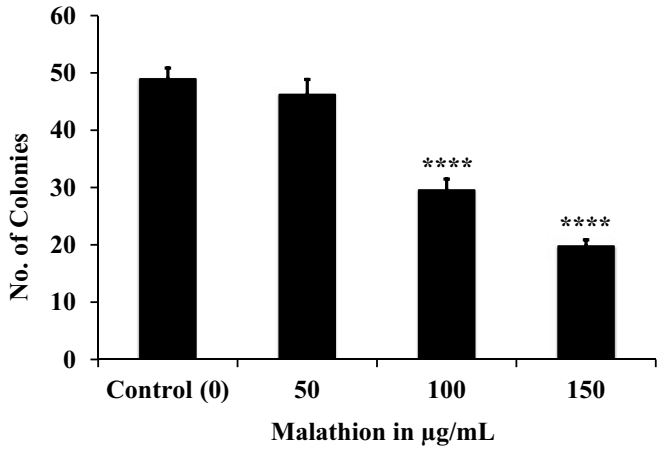

Fig. 2. Effect of malathion on human lymphocytes colony formation. Values without the symbol (*) is not significant compared to control at $p > 0.05$; significant compared to control at ****$p < 0.0001$ level.

value = CT value of target gene – CT value of reference gene and ΔΔCT value = ΔCT value of the test for target gene – ΔCT value of control for the target gene. The relative gene expression (RQ) is expressed as $2^{-\Delta\Delta CT}$.

3. Results

Treatment of human lymphocytes with different concentrations of malathion caused a concentration-dependent decrease in lymphocyte viability. At the lowest concentration of malathion treatment (50 µg/mL), human lymphocytes were viable, indicating that a low concentration of malathion is not cytotoxic (Fig. 1). Similarly, -the lowest concentration of malathion treated lymphocytes recorded more colonies compared to 100 and 150 µg/mL malathion treated lymphocytes. The number of colonies in control and 50 µg/mL malathion treated groups are not having any significant differences ($p > 0.05$; Fig. 2).

Microarray analysis revealed the presence of differentially regulated genes in low concentration of malathion treated human lymphocytes. Among the 4388 genes which were differentially expressed, 659 genes (15%) were up regulated and 3729 genes (85%) were down regulated (Fig. 3). About 57 differentially regulated genes linked to cancer are given in Table 2. The functionally explored groups include the genes

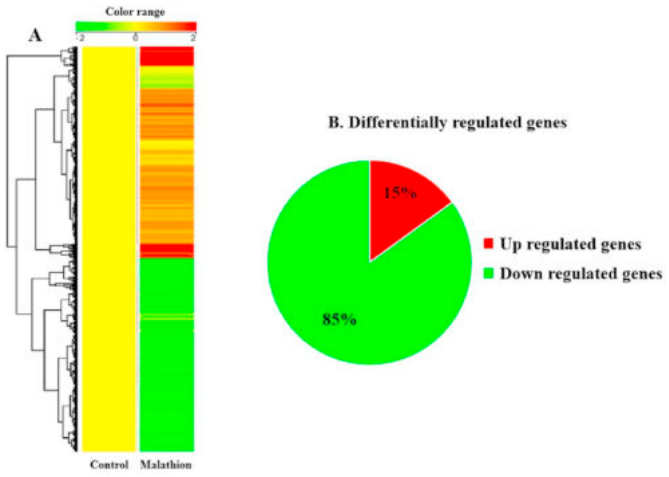

Fig. 3. (A) An overview of cluster of differentially regulated genes in malathion treated sample (50 µg/mL) compared to control. Yellow: Control (neutral); Green: Down regulation; Red: Up regulation. (B) Percentage of differentially regulated genes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

related to the growth and differentiation of B and T cells, immunoglobulin production, haematopoiesis, tumor suppressors, oncogenes, and those involved in the signal transduction pathways like MAPK and RAS. The significant relative over expression of *LPCAT4, MICA,* and *BMI1* genes (Fig. 4a, b, c) and low-level expression of *MAP2K3* and *HLA-E* genes were observed in malathion treated human lymphocytes compared to control group (Fig. 4d and e). This differential relative gene expression is dose-dependent (Fig. 4).

4. Discussion

This study highlights the identification of differentially regulated genes involved in carcinogenesis, upon exposure of malathion pesticide to human lymphocytes. Differential expression of cancer related genes identified in malathion treated human lymphocytes (Table 2) proved that the low concentration of malathion induces the expression of cancer genes without causing cytotoxicity (Fig. 1) and promotes the lymphocyte proliferation (Fig. 2). From this, it can be inferred that low concentration of malathion is enough to show its carcinogenic nature in human lymphocytes.

4.1. B and T cells

Differentially regulated genes in the growth and differentiation of B and T cells: *CEBPG, MICA*, *PAX1,* and *TFRC* were up regulated and the *HLA-A, HLA-E* and *LGALS1* were down regulated (Table 2) when human lymphocytes were exposed to malathion pesticide and may lead to the development of hematological cancers. *CEBPG* is involved in the differentiation of B cells. It has been shown that in acute myeloid leukemia samples *CEBPG* expression was drastically up regulated (Alberich-Jorda et al., 2012). *TFRC* is involved in the positive regulation of B and T cell proliferation and their activation. Up regulation of *TFRC* genes in this study (Table 2), suggesting for the uncontrolled proliferation of B and T cells. *TFRC* is also involved in the cellular iron uptake and haematopoiesis, and it's over expression was reported in hepatocellular carcinoma (Kindrat et al., 2016). *LGALS1* is involved in the activation, regulation- and differentiation of B cells. In acute myeloid leukemia, the expression of *LGALS1* was down regulated (El-Leithy et al., 2015). *MICA* and *PAX1* help in the T cell activation and its regulation. The relative over expression of *MICA* upon malathion treatment to human lymphocytes (Fig. 4b, Table 2) and it is reported to promote tumor immune evasion in tumor cells by down regulating *NKG2*, a receptor for natural killer cells, and thus affecting the anti-tumor effect of natural killer and cytotoxic T cells (Chen and Gyllensten, 2014). *PAX1* is a potential biomarker for cervical cancer (Kan et al., 2014). *HLA-A* and *HLA-E* are involved in the positive regulation of CD8-positive, α, and β-T cell proliferation. *HLA-A* helps in the activation of memory T cells and its positive regulation. Low level expression of *HLA-E* gene upon malathion pesticide exposure to human lymphocytes was found in this study (Fig. 4e, Table 2) and has been reported to increase the risk of cancer as their down regulation lead to the loss of protective anti-tumor activity mediated by T cells (Hicklin et al., 1999; Marin et al., 2003; Kaneko et al., 2011).

4.2. Immunoglobulin production

Genes involved in the production and regulation of immunoglobulin *IL13RA2, MICA, TFRC, TLR8,* and *VPREB3,* were up regulated and *C1QBP, HLA-A,* and *HLA-E* are down regulated in the malathion treated human lymphocytes (Table 2). *C1QBP, HLA-A, HLA-E, MICA* and *TLR8* help in the adaptive immune response based on the somatic recombination of immune receptors built from immunoglobulin superfamily domains. The expression of *C1QBP* was significantly decreased in the tissues of human cervical squamous cell carcinoma (Liu et al., 2012). It has been reported in renal carcinoma cells, where its down regulation has resulted in the elevation of cell adhesion and metastasis

Table 2
Malathion induced cancer linked genes in human lymphocytes.

UniGene	Symbol	Description	Differential gene expression	Fold change
Hs.272011	*B4GALT1*	UDP-Gal:β-GlcNAc β 1,4- galactosyltransferase, polypeptide 1	↓	−1.804
Hs.380403	*BMI1*	BMI1 polycomb ring finger oncogene	↑	7.255
Hs.255935	*BTG1*	B-cell translocation gene 1, anti-proliferative	↓	−1.784
Hs.555866	*C1QBP*	Complement component 1, q subcomponent binding protein	↓	−7.207
Hs.138378	*CASP4*	Caspase 4, apoptosis-related cysteine peptidase	↓	−1.804
Hs.460988	*CBFB*	Core-binding factor, beta subunit	↑	4.827
Hs.163867	*CD14*	CD14 molecule	↓	−6.421
Hs.58685	*CD5*	CD5 molecule	↓	−1.753
Hs.120949	*CD36*	CD36 molecule	↑	4.134
Hs.19192	*CDK2*	Cyclin-dependent kinase 2	↑	4.067
Hs.429666	*CEBPG*	CCAAT/enhancer binding protein (C/EBP), gamma	↑	2.569
Hs.489142	*COL1A2*	Collagen, type 1, alpha 2	↑	5.188
Hs.443960	*DDX11*	DEAD/H (Asp-Glu-Ala-Asp/His) box polypeptide 11	↑	5.733
Hs.171695	*DUSP1*	Dual specificity phosphatase 1	↓	−1.746
Hs.371218	*EPHA4*	EPH receptor A4	↑	2.287
Hs.438862	*EPS8L1*	EPS8-like 1	↑	3.623
Hs.166015	*FGF6*	Fibroblast growth factor 6	↑	4.551
Hs.264887	*FGFR1*	Fibroblast growth factor receptor 1	↑	4.571
Hs.584654	*FOXO4*	Forkhead box O4	↑	3.629
Hs.1428	*FSHR*	Follicle stimulating hormone receptor	↑	5.124
Hs.77269	*GNAI2*	Guanine nucleotide binding protein (G protein), alpha inhibiting activity polypeptide 2	↓	−6.958
Hs.438219	*GPS2*	G protein pathway suppressor 2	↓	−3.355
Hs.475150	*GRAMD4*	GRAM domain containing 4	↓	−1.784
Hs.523836	*GSTP1*	Glutathione S-transferase pi 1	↓	−5.150
Hs.181244	*HLA-A*	Major histocompatibility complex, class I, A	↓	−5.197
Hs.650174	*HLA-E*	Major histocompatibility complex, class I, E	↓	−7.528
Hs.90753	*HTATIP2*	HIV-1 Tat interactive protein 2, 30 kDa	↓	−1.804
Hs.336046	*IL13RA2*	Interleukin 13 receptor, alpha-2	↑	4.195
Hs.522819	*IRAK1*	Interleukin-1 receptor associated kinase 1	↑	4.754
Hs.445351	*LGALS1*	Lectin, galactoside-binding, soluble, 1	↓	−5.698
Hs.352614	*LPCAT4*	Lysophosphatidylcholine acyltransferase 4	↑	15.569
Hs.523221	*LZTS2*	Leucine zipper, putative tumor suppressor 2	↓	−3.349
Hs.514012	*MAP2K3*	Mitogen-activated protein kinase 3	↓	−7.863
Hs.130838	*MICA*	MHC class I polypeptide-related sequence A	↑	7.745
Hs.494457	*NINJ1*	Ninjurin 1	↓	−6.599
Hs.463456	*NME2*	Non-metastatic cells 2, protein (NM23B)	↓	−6.158
Hs.494312	*NTRK2*	Neurotrophic tyrosine kinase, receptor, type 2	↑	6.673
Hs.349082	*PAX1*	Paired box 1	↑	3.238
Hs.433863	*PEBP1*	Phosphatidylethanolamine binding protein 1	↓	−5.554
Hs.494691	*PFN1*	Profilin 1	↓	−5.825
Hs.514303	*PHB*	Prohibitin	↓	−1.763
Hs.77274	*PLAU*	Plasminogen activator, urokinase	↑	5.656
Hs.526464	*PML*	Promyelocytic leukemia	↓	−4.349
Hs.508999	*PRKD1*	Protein kinase D1	↑	3.182
Hs.531642	*RAB11FIP3*	RAB11 family interacting protein 3 (class II)	↑	3.742
Hs.433795	*SHC1*	SHC (Src homology 2 domain containing) transforming protein 1	↑	2.096
Hs.527973	*SOCS3*	Suppressor of cytokine signaling 3	↓	−4.090
Hs.89640	*TEK*	TEK tyrosine kinase, endothelial	↑	6.287
Hs.529618	*TFRC*	Transferrin receptor	↑	2.816
Hs.660543	*TLR8*	Toll-like receptor 8	↑	2.867
Hs.279640	*TPR*	Translocated promoter region	↑	1.808
Hs.104223	*TRIM35*	Tripartite motif containing 35	↓	−1.753
Hs.517981	*TUSC2*	Tumor suppressor candidate 2	↓	−3.490
Hs.66744	*TWIST1*	Twist homolog 1 (Drosophila)	↑	4.023
Hs.517792	*VHL*	von Hippel-Lindau tumor suppressor	↓	−2.484
Hs.136713	*VPREB3*	Pre-B lymphocyte 3	↑	2.924
Hs.591980	*WT1*	Wilms tumor 1	↑	5.933

↑ - Up regulated expression; ↓ - Down regulated expression of the gene with respect to the control.

(Wang et al., 2017). *IL13RA2* negatively regulates immunoglobulin production and is highly expressed in human glioblastoma multiform and squamous cell carcinoma of the head and neck (SCCHN) cell lines (Kawakami et al., 2003; Newman et al., 2017). Grimmig et al. (2015) have reported that during the pathogenesis of pancreatic cancer, *TLR8* was over expressed and its signaling lead to tumor cell proliferation and chemoresistance.

4.3. Haematopoiesis

The genes associated with haematopoiesis *BMI1, CBFB, CD36, CEBPG, FSHR, PAX1, TEK* and *TFRC* were up regulated and *CD14, LGALS1* and *NME2* were down regulated in the malathion treated lymphocytes (Table 2). The overexpression of the polycomb group *BMI1* gene was found in this study(Fig. 4c) and this gene regulates cell proliferation and is a molecular marker for predicting prognosis of chronic myeloid leukemia (Mohty et al., 2007). *CBFB* expression was elevated in prostate and ovarian cancer cells, and its knockout resulted in the inhibition of anchorage independent growth of the cancer cells. Thus, this gene is essential for malignancy and - promotes carcinogenesis (Davis et al., 2010). In adrenocortical carcinoma, a significant reduction in the expression of *CD14* was reported by Kanczkowski et al. (2010). *CD36* was expressed in 97% of ovarian cancers (Wang et al., 2016a; Wang et al., 2016b), and has been reported as a potential

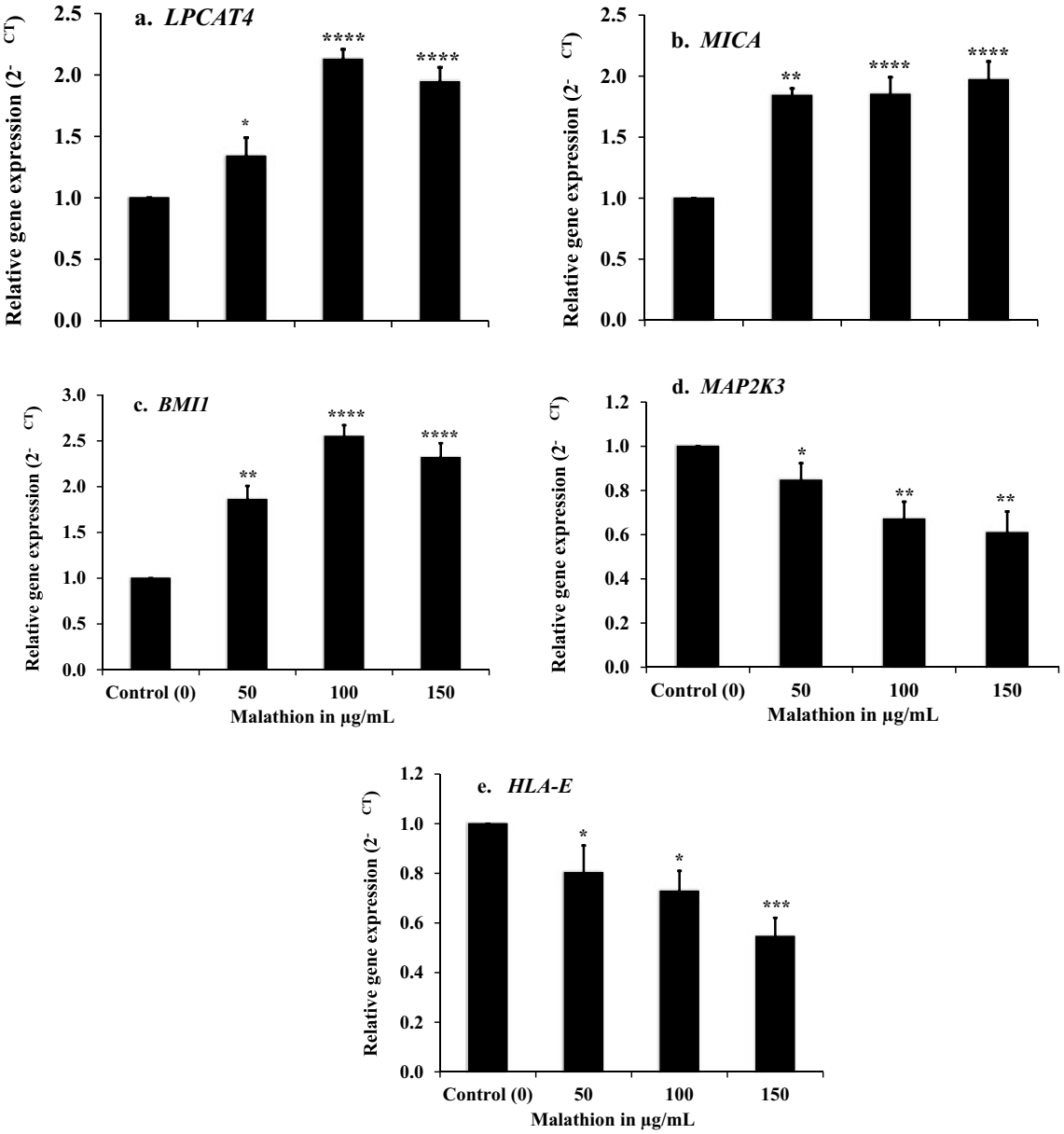

Fig. 4. Malathion induced gene expression in human lymphocytes validated by qRT-PCR.

prognostic biomarker in cancer (Enciu et al., 2018). *FSHR* was over expressed in numerous metastatic tumors (Siraj et al., 2013). The down regulation of *NME2* leads to metastasis as it was reported to reduce proliferation, migration and invasion of gastric cancer cells to limit metastasis (Liu et al., 2015). *TEK*, also known as *TIE2*, helps in angiogenesis along with the growth factors angiopoietin 1 and 2 (Jones and Dumont, 2000). Its high expression can lead to breast cancer metastasis, particularly bone invasion (Dales et al., 2004; Min et al., 2010).

4.4. Tumor suppressor genes

Tumor suppressor genes like *B4GALT1, BTG1, CASP4, CD5, DUSP1, GPS2, GSTP1, GRAMD4, HTATIP2, LZTS2, NINJ1, PFN1, PHB, PML, SOCS3, TRIM35, TUSC2* and *VHL* were down regulated in this study (Table 2). The glycogene *B4GALT1* is down regulated in colorectal cancer and frequently methylated (Poeta et al., 2012). Also, *BTG1* was down regulated in ovarian carcinoma by promoter methylation (Kim et al., 2017). Notably, *CASP4* expression is suppressed in head and neck squamous cell carcinoma (Li et al., 2003, 2004). *CD5* acts as a shield for cancer cells (Dalloul, 2009); down regulation of this gene can disrupt the protective barrier and promote carcinogenesis. *DUSP1* in prostate cancer and *GPS2* in liposarcoma were significantly down regulated (Rauhala et al., 2005; Huang et al., 2016). In prostate cancer, *GSTP1*, which codes for a detoxification enzyme, was largely down regulated (Beer et al., 2002). The high expression of death inducing protein *GRAMD4* normally increases apoptosis in cancer cells (Deng and Wu, 2000), but, in this study, it is down regulated (Table 2), thus, may be enhancing cancer cell survival. *HTATIP2* acts as a tumor suppressor, decreasing invasion and motility of malignant cells, inhibiting epithelial-mesenchymal transition and by enhancing apoptosis (Li et al., 2009; Tong et al., 2009; Chen et al., 2010; Liu and Yang, 2011; Guo et al., 2014; Zhu et al., 2014). In primary gliomas, *HTATIP2* expression was absent or decreased compared to the normal brain tissue (Dong et al., 2015). The down regulation of its expression results in the protection of cancer cells from apoptosis (Chen and Shtivelman, 2010). *LZTS2* is a tumor suppressor gene, inhibiting cell proliferation and G1/S cell cycle transition; a significant reduction of *LZTS2* expression in non-small cell lung cancer, enhancing its progression (Cui et al., 2013). By inhibiting

the IL-6 signaling pathway, *NINJ1* was reported to suppress migration, invasion, and metastasis of lung cancer (Jang et al., 2016), so if down regulated can pave the way for the emergence of cancer. *PFN1* is a tumor suppressor gene, and leads to the development of cancer when it is down regulated, and was confirmed in the case of pancreatic cancer (Yao et al., 2014). Down regulation of *PHB* expression has been noted in gliomas (Nijtmans et al., 2000). *PML* expression was reduced or lost in the pathogenesis of prostate and breast cancers (Gurrieri et al., 2004). The expression of *SOCS3*, one of the most significant members of the *SOCS* family, was down regulated in colorectal cancer (Chu et al., 2017). *TRIM35* is a tumor suppressor gene and was down regulated in many cancers (Zhao and Zhao, 2016). In lung cancer, over expression of *TUSC2* was found to decrease cell growth by inducing G1 cell cycle arrest and promoting apoptosis (Kondo et al., 2001; Li et al., 2014). Down regulation of *TUSC2* gene can result in the development of cancers. Inactivation of *VHL* was responsible for most sporadic renal cell carcinomas (Latif et al., 1993) and thus, down regulation of *VHL* (Table 2) can result in carcinogenesis.

4.5. Oncogenes

BMI1, DDX11, FOXO4, NTRK2, PLAU, SHC1, TEK, TWIST1 and *WT1* oncogenes up regulated in this study (Table 2) indicating that malathion induces the genes responsible for promoting carcinogenesis. *BMI1* (Fig. 4c), and *TWIST1* have been reported to enhance metastasis and chemoresistance, thus promoting carcinogenesis (Ren et al., 2016). The drastic over expression of helicase *DDX11* was reported in primary and metastatic melanoma (Bhattacharya et al., 2012). Like the members of Forkhead Box Class O protein family, over expression of *FOXO4* has been reported to promote metastasis and thus the progression of cancer (Kim et al., 2018). *NTRK2* is an oncogene and when it was up regulated, there was an increase of TrkB protein that resulted in enhanced proliferation and invasion of cancer cells (Hu et al., 2016). High expression of *PLAU*,encodes urokinase plasminogen activator (uPA), was reported in breast cancer cells (Pavet et al., 2014). *SHC* helps in the regulation of cell adhesion and motility in breast cancer by interacting with a5b1 integrin (Mauro et al., 1999). *WT1* is over expressed in different types of cancers, and it's knockdown resulted in mitochondrial damage that had caused the inhibition of malignant cell growth (Tatsumi et al., 2008).

4.6. MAPK pathway

Genes associated with MAPK pathway *CD36, EPHA4, FGF6*, *FGFR1, FSHR, IRAK1, NTRK2, SHC1, TLR8* and *TPR* were up regulated, and *GNAI2, GSTP1, MAP2K3* (Fig. 4d) and *PEBP1* were down regulated in malathion treated human lymphocytes (Table 2). *EPHA4* has been reported to promote the proliferation and migration of cancer cells through *EPHA4-FGFR1* signaling pathway (Fukai et al., 2008). *FGF* signaling network plays a ubiquitous role in normal growth, survival, and differentiation of cells, and also in angiogenesis (Korc and Friesel, 2009). *FGF6* was over expressed in prostatic intraepithelial neoplasia and prostate cancer, and promotes the proliferation of the transformed prostatic epithelial cells (Ropiquet et al., 2000). Zhang et al. (2009) found that there was an over expression of *FGFR* in ovarian epithelial cancer. Elevated expression of *FGFR1* was detected in non-small cell lung cancer, which was responsible for promoting angiogenesis (Pu et al., 2017). *IRAK1* has been reported to play an important role in the pathogenesis of multiple cancers; over expression of this gene has resulted in the development of hepatocellular carcinoma (Ye et al., 2017). *TPR* is involved in the spatial and temporal regulation of spindle checkpoints and ensures the proper recruitment of checkpoint proteins for the proper formation of anaphase (Nakano et al., 2010). Up regulation of *TPR* support malignant transformations (Lee et al., 2006). *GNAI2* functions as a metastasis suppressor and the knockdown of its expression has resulted in the enhanced migration and invasion of hepatocellular carcinoma cells (Yao et al., 2010). The expression of *MAP2K3* produces senescence protein and its down regulation (Fig. 4d) has been reported to provides immortality to breast cancer cells (Jia et al., 2010). *PEBP1* is a metastasis suppressor gene in breast cancer and its down regulation promotes metastasis, thus, paving the way for the pathology of cancer (Hagan et al., 2005).

4.7. RAS pathway

Genes associated with RAS pathway involved in carcinogenesis *CDK2*, *COLIA2*, *EPS8L1*, *FGF6*, *FGFR1*, *FOXO4, LPCAT4 (*Fig. 4a*), PRKD1*, RAB11FIP3*, SHC1* and *TEK* which were up regulated, and *PFN1* was down regulated in malathion treated human lymphocytes (Table 2). A study showed that *CDK2* is functionally associated with hyperproliferation in multiple cancer cells and its expression level was elevated in human cholangiocarcinoma tissues (Zheng et al., 2016) and induces radio resistance in glioblastoma (Wang et al., 2016a,Wang et al., 2016b). *COL1A2* over expression promotes invasion and metastasis of gastric cancer cells (Yasui et al., 2004). The gene *EPS8L1* was over expressed in human pituitary tumors and it led to the amplification of growth factor receptor signaling, promoting proliferation and survival of the cells (Xu et al., 2008). The elevated level of *LPCAT4* gene expression in malathion treated human lymphocytes (Fig. 4a) and was also elevated in human colorectal cancer (Kurabe et al., 2013). *PRKD1* was up regulated in pancreatic adenocarcinoma and promoted the survival of the cells and oncogenic signaling (Storz and Doeppler, 2014). Increased expression of RAB11FIP3 in papillary thyroid cancer and breast cancer plays a major role in endocytotic recycling and through the transport of EGFR promotes tumorigenesis (Tong et al., 2017; Liu et al., 2018).

5. Conclusion

This study evidences carcinogenic nature of malathion. The low concentration of this pesticide is not cytotoxic and it promotes the proliferation of lymphocytes. Malathion induced differentially regulated genes are involved in the activation and proliferation of B and T cells, immunoglobulin production and regulation, haematopoiesis and in the signal transduction pathways like MAPK, RAS, tumor suppression and oncogenes. These differentially regulated genes are evident in the etiology of cancer and also act as prognostic markers. A low concentration of malathion pesticide is enough to pave the way for the initiation, progression and pathogenesis of cancer.

Declaration of competing interest

None declared.

Acknowledgements

We are thankful to the authorities of Central University of Kerala for providing facilities and Kerala State Council for Science, Technology and Environment (KSCSTE) for the Research fellowships. We also thank UGC, India (No.F.30–25/2014(BSR)/27-Jan-2016), DBT, India (6242-P-36/RGCB/PMD/DBT/HPGS/2015/29-July-2015) and DST-SERB, India (SB/YS/LS-387/2013/31-Oct-2014) for funding the research projects. Thankful to Genotypic Technologies Pvt. Ltd Bengaluru for the Microarray platform. We express our sincere gratitude to Dr. V. Vasudev, Former Professor, University of Mysore, Karnataka, India, and Professor M. Arunachalam, Department of Zoology, Central University of Kerala, Periya, India, for their critical review of the manuscript and encouragement.

References

Agarwal, A., et al., 2015. Pesticide residue in water - a challenging task in India. Environ. Monit. Assess. 187 (2), 54.

Akbel, E., et al., 2018. The subchronic exposure to malathion, an organophosphate pesticide, causes lipid peroxidation, oxidative stress, and tissue damage in rats: the protective role of resveratrol. Toxicology Research 7 (3), 503–512.

Alberich-Jorda, M., et al., 2012. C/EBPγ deregulation results in differentiation arrest in acute myeloid leukemia. J. Clin. Investig. 122 (12), 4490–4504.

ATSDR (Agency for Toxic Substances and Disease Registry), 2003. Toxicological profile for malathion. Accessed from. https://www.atsdr.cdc.gov/ToxProfiles/tp.asp?id=522&tid=92.

Beer, T.M., et al., 2002. Polymorphisms of *GSTP1* and related genes and prostate cancer risk. Prostate Cancer Prostatic Dis. 5 (1), 22.

Bhattacharya, C., et al., 2012. The DEAD/DEAH box helicase, *DDX11*, is essential for the survival of advanced melanomas. Mol. Cancer 11 (1), 82.

Bonner, M.R., et al., 2007. Malathion exposure and the incidence of cancer. Am. J. Epidemiol. 166 (9), 1023–1034.

Cabello, G., et al., 2001. A rat mammary tumor model induced by the organophosphorous pesticides parathion and malathion, possibly through acetylcholinesterase inhibition. Environ. Health Perspect. 109, 471–479.

Chen, D., Gyllensten, U., 2014. *MICA* polymorphism: biology and importance in cancer. Carcinogenesis 35 (12), 2633–2642.

Chen, V., Shtivelman, E., 2010. *CC3/TIP30* regulates metabolic adaptation of tumor cells to glucose limitation. Cell Cycle 9 (24), 4941–4953.

Chen, X., et al., 2010. Expression of *TIP30* tumor suppressor gene is down-regulated in human colorectal carcinoma. Dig. Dis. Sci. 55 (8), 2219–2226.

Chu, Q., et al., 2017. Prognostic significance of *SOCS3* and its biological function in colorectal cancer. Gene 627, 114–122.

Crowley, L.C., Waterhouse, N.J., 2016. Measuring survival of hematopoietic cancer cells with the colony-forming assay in soft agar. Cold Spring Harb. Protoc. 2016 (8), 725–729.

Cui, Q.Z., et al., 2013. Leucine zipper tumor suppressor 2 inhibits cell proliferation and regulates Lef/Tcf-dependent transcription through Akt/GSK3β signaling pathway in lung cancer. J. Histochem. Cytochem. 61 (9), 659–670.

Dales, J.P., et al., 2004. Prediction of metastasis risk (11-year follow-up) using VEGF-R1, VEGF-R2, Tie-2/Tek and CD105 expression in breast cancer (n= 905). Br. J. Canc. 90 (6), 1216.

Dalloul, A., 2009. CD5: a safeguard against autoimmunity and a shield for cancer cells. Autoimmun. Rev. 8 (4), 349–353.

Davis, J.N., et al., 2010. Association of core-binding factor β with the malignant phenotype of prostate and ovarian cancer cells. J. Cell. Physiol. 225 (3), 875–887.

Deng, Y., Wu, X., 2000. Peg3/Pw1 promotes p53-mediated apoptosis by inducing Bax translocation from cytosol to mitochondria. Proc. Natl. Acad. Sci. 97 (22), 12050–12055.

Dong, X., et al., 2015. Downregulation of *HTATIP2* expression is associated with promoter methylation and poor prognosis in glioma. Exp. Mol. Pathol. 98 (2), 192–199.

El-Leithy, A.A., et al., 2015. Expression profiling of cancer-related galectins in acute myeloid leukemia. Tumor Biol. 36 (10), 7929–7939.

Enciu, A.M., et al., 2018. Targeting *CD36* as biomarker for metastasis prognostic: how far from translation into clinical practice? BioMed Res. Int. 12 2018.

Finney, D.J., 1971. Probit Analysis, third ed. Cambridge University Press, Cambridge.

Fukai, J., et al., 2008. *EphA4* promotes cell proliferation and migration through a novel EphA4-FGFR1 signaling pathway in the human glioma U251 cell line. Mol. Cancer Ther. 7 (9), 2768–2778.

Goodson III, W.H., et al., 2015. Assessing the carcinogenic potential of low-dose exposures to chemical mixtures in the environment: the challenge ahead. Carcinogenesis 36 (1), S254–S296.

Grimmig, T., et al., 2015. *TLR7* and *TLR8* expression increases tumor cell proliferation and promotes chemoresistance in human pancreatic cancer. Int. J. Oncol. 47 (3), 857–866.

Guo, S., et al., 2014. Decreased *TIP30* expression predicts poor prognosis in pancreatic cancer patients. Int. J. Cancer 134 (6), 1369–1378.

Gurrieri, C., et al., 2004. Loss of the tumor suppressor PML in human cancers of multiple histologic origins. J. Natl. Cancer Inst. 96 (4), 269–279.

Gurushankara, H.P., et al., 2007a. Effect of malathion on survival, growth, and food consumption of Indian cricket frog (*Limnonectus limnocharis*) tadpoles. Arch. Environ. Contam. Toxicol. 52 (2), 251–256.

Gurushankara, H.P., et al., 2007b. Impact of malathion on lipid metabolism in *Limnonectus limnocharis*. Pestic. Biochem. Physiol. 88 (1), 50–56.

Hagan, S., et al., 2005. Reduction of Raf-1 kinase inhibitor protein expression correlates with breast cancer metastasis. Clin. Cancer Res. 11 (20), 7392–7397.

Harper Jr., W.F., et al., 2017. Adsorption of malathion onto copper and iron surfaces relevant to water infrastructure. J. Am. Water Work. Assoc. 109 (11), 494–502.

Hanahan, D., Weinberg, R.A., 2000. The hallmarks of cancer. Cell 100 (1), 57–70.

Hicklin, D.J., et al., 1999. HLA class I antigen downregulation in human cancers: T-cell immunotherapy revives an old story. Mol. Med. Today 5 (4), 178–186.

Hu, J., et al., 2016. *NTRK2* is an oncogene and associated with microRNA-22 regulation in human gastric cancer cell lines. Tumor Biol. 37 (11), 15115–15123.

Huang, X.D., et al., 2016. G protein pathway suppressor 2 (*GPS2*) acts as a tumor suppressor in liposarcoma. Tumor Biol. 37 (10), 13333–13343.

IARC (International Agency for Research on Cancer), 2017. Some organophosphate insecticides and herbicides: Malathion. Accessed from. https://monographs.iarc.fr/iarc-monographs-on-the-evaluation-of-carcinogenic-risks-to-humans-4/.

Jang, Y.S., et al., 2016. Ninjurin1 suppresses metastatic property of lung cancer cells through inhibition of interleukin 6 signaling pathway. Int. J. Cancer 139 (2), 383–395.

Jia, M., et al., 2010. Proteome profiling of immortalization-to-senescence transition of human breast epithelial cells identified *MAP2K3* as a senescence-promoting protein which is downregulated in human breast cancer. Proteom. Clin. Appl. 4 (10-11), 816–828.

Jones, N., Dumont, D.J., 2000. Tek/Tie2 signaling: new and old partners. Cancer Metastasis Rev. 19 (1–2), 13–17.

Kalender, S., et al., 2010. Malathion induced hepatotoxicity in rats: the effects of vitamins C and E. Food Chem. Toxicol. 48, 633–638.

Kan, Y.Y., et al., 2014. Methylation as a potential biomarker for cervical cancer screening. Int. J. Gynecol. Cancer 24 (5), 928–934.

Kanczkowski, W., et al., 2010. Abrogation of *TLR4* and *CD14* expression and signaling in human adrenocortical tumors. J. Clin. Endocrinol. Metab. 95 (12), E421–E429.

Kaneko, K., et al., 2011. Clinical implication of *HLA* class I expression in breast cancer. BMC Canc. 11 (1), 454.

Kawakami, M., et al., 2003. Interleukin-13 receptor α2 chain in human head and neck cancer serves as a unique diagnostic marker. Clin. Cancer Res. 9 (17), 6381–6388.

Kim, C.G., et al., 2018. Role of Forkhead Box Class O proteins in cancer progression and metastasis. Semin. Cancer Biol. 50, 142–151.

Kim, J.Y., et al., 2017. B-cell translocation gene 1 is downregulated by promoter methylation in ovarian carcinoma. J. Cancer 8 (14), 2669.

Kindrat, I., et al., 2016. MicroRNA-152-mediated dysregulation of hepatic transferrin receptor 1 in liver carcinogenesis. Oncotarget 7 (2), 1276–1287.

Kondo, M., et al., 2001. Overexpression of candidate tumor suppressor gene *FUS1* isolated from the 3p21. 3 homozygous deletion region leads to G1 arrest and growth inhibition of lung cancer cells. Oncogene 20 (43), 6258.

Korc, M., Friesel, R.E., 2009. The role of fibroblast growth factors in tumor growth. Curr. Cancer Drug Targets 9 (5), 639–651.

Kurabe, N., et al., 2013. Accumulated phosphatidylcholine (16: 0/16: 1) in human colorectal cancer; possible involvement of *LPCAT4*. Cancer Sci. 104 (10), 1295–1302.

Latif, F., et al., 1993. Identification of the von Hippel-Lindau disease tumor suppressor gene. Science 260 (5112), 1317–1320.

Lee, S., et al., 2006. Differential expression in normal-adenoma-carcinoma sequence suggests complex molecular carcinogenesis in colon. Oncol. Rep. 16 (4), 747–754.

Lerro, C.C., et al., 2015. Organophosphate insecticide use and cancer incidence among spouses of pesticide applicators in the Agricultural Health Study. Occup. Environ. Med. 72 (10), 736–744.

Li, H., et al., 2003. Expression and correlation of apoptosis-related gene *c-IAP2* and caspase-4 in head and cervical undifferentiation squamous cell carcinoma. J. Clin. Otorhinolaryngol. 17 (12), 739–741.

Li, H., et al., 2004. Expression and correlation of apoptosis-related gene *c-IAP2* and caspase-4 in sinonasal squamous carcinoma. Chinese J. Otorhinolaryngol. Head Neck Surg. 39 (6), 324–327.

Li, L., et al., 2014. Synergistic effects of eukaryotic coexpression plasmid carrying *LKB1* and *FUS1* genes on lung cancer in vitro and in vivo. J. Cancer Res. Clin. Oncol. 140 (6), 895–907.

Li, X., et al., 2009. Reduction of *TIP30* correlates with poor prognosis of gastric cancer patients and its restoration drastically inhibits tumor growth and metastasis. Int. J. Cancer 124 (3), 713–721.

Liu, D.C., Yang, Z.L., 2011. Clinicopathologic significance of minichromosome maintenance protein 2 and Tat-interacting protein 30 expression in benign and malignant lesions of the gallbladder. Hum. Pathol. 42 (11), 1676–1683.

Liu, Y., et al., 2018. *Eps15* homology domain 1 promotes the evolution of papillary thyroid cancer by regulating endocytotic recycling of epidermal growth factor receptor. Oncology Letters 16 (4), 4263–4270.

Liu, Y.F., et al., 2015. *NME2* reduces proliferation, migration and invasion of gastric cancer cells to limit metastasis. PLoS One 10 (2), e0115968.

Liu, Z., et al., 2012. The role of the globular heads of C1q receptor (*gC1qR*) gene in regulating apoptosis of human cervical squamous cell carcinoma. Cell. Physiol. Biochem. 30 (5), 1181–1190.

Marin, R., et al., 2003. Analysis of *HLA-E* expression in human tumors. Immunogenetics 54 (11), 767–775.

Mauro, L., et al., 1999. SHC–α5β1 integrin interactions regulate breast cancer cell adhesion and motility. Exp. Cell Res. 252 (2), 439–448.

Min, Y., et al., 2010. Tie2 signaling regulates osteoclastogenesis and osteolytic bone invasion of breast cancer. Cancer Res. 70 (7), 2819–2828.

Mitra, P.S., et al., 2012. Analysis of the toxicogenomic effects of exposure to persistent organic pollutants (POPs) in Slovakian girls: correlations between gene expression and disease risk. Environ. Int. 39 (1), 188–199.

Mohty, M., et al., 2007. The polycomb group *BMI1* gene is a molecular marker for predicting prognosis of chronic myeloid leukemia. Blood 110 (1), 380–383.

Moore, P.D., et al., 2010. Malathion- induced oxidative stress, cytotoxicity, and genotoxicity in human liver carcinoma (HepG2) cells. Environ. Toxicol. 25 (3), 221–226.

Muniz, J.F., et al., 2008. Biomarkers of oxidative stress and DNA damage in agricultural workers: a pilot study. Toxicol. Appl. Pharmacol. 227, 97–107.

Nain, S., et al., 2011. Immunotoxicity and disease resistance in Japanese quail (*Coturnix coturnix japonica*) exposed to malathion. Ecotoxicology 20, 892–900.

Nakano, H., et al., 2010. Nucleoporin translocated promoter region (Tpr) associates with dynein complex, preventing chromosome lagging formation during mitosis. J. Biol. Chem. 285 (14), 10841–10849.

Navarrete-Meneses, M.P., et al., 2018. Low concentrations of permethrin and malathion induce numerical and structural abnormalities in *KMT2A* and *IGH* genes in vitro. J. Appl. Toxicol. 38 (9), 1262–1270.

Newman, J.P., et al., 2017. Interleukin-13 receptor alpha 2 cooperates with EGFRvIII signaling to promote glioblastoma multiforme. Nat. Commun. 8 (1), 1913.

Nijtmans, L.G., et al., 2000. Prohibitins act as a membrane-bound chaperone for the stabilization of mitochondrial proteins. EMBO J. 19 (11), 2444–2451.

Pavet, V., et al., 2014. Plasminogen activator urokinase expression reveals TRAIL responsiveness and supports fractional survival of cancer cells. Cell Death Dis. 5 (1), e1043.

Poeta, M.L., et al., 2012. Aberrant promoter methylation of beta-1, 4 galactosyltransferase 1 as potential cancer specific biomarker of colorectal tumors. Genes Chromosomes Cancer 51 (12), 1133–1143.
Pu, D., et al., 2017. Fibroblast growth factor receptor 1, partly related to vascular endothelial growth factor receptor 2 and microvessel density, is an independent prognostic factor for non-small cell lung cancer. Med. Sci. Monit.: International Medical Journal of Experimental and Clinical Research 23, 247.
Rao, A.S., Pillala, R.R., 2001. The concentration of pesticides in sediments from Kolleru Lake in India. Pest Manag. Sci. 57, 620–624.
Rauhala, H.E., et al., 2005. Dual-specificity phosphatase 1 and serum/glucocorticoid-regulated kinase are downregulated in prostate cancer. Int. J. Cancer 117 (5), 738–745.
Ren, H., et al., 2016. *TWIST1* and *BMI1* in cancer metastasis and chemoresistance. J. Cancer 7 (9), 1074.
Reuber, M.D., 1985. Carcinogenicity and toxicity of malathion and malaoxon. Environ. Res. 37 (1), 119–153.
Ropiquet, F., et al., 2000. Increased expression of fibroblast growth factor 6 in human prostatic intraepithelial neoplasia and prostate cancer. Cancer Res. 60, 4245–4250.
Selmi, S., et al., 2018. Malathion, an organophosphate insecticide, provokes metabolic, histopathologic and molecular disorders in liver and kidney in prepubertal male mice. Toxicology Reports 5, 189–195.
Siraj, A., et al., 2013. Expression of follicle-stimulating hormone receptor by the vascular endothelium in tumor metastases. BMC Canc. 13 (1), 246.
Storz, P., Doeppler, H., 2014. Upregulation of *PRKD1* gene expression by activated K-ras in pancreatic cancer cells (609.20). FASEB J. 28 (1), 609–620.
Tatsumi, N., et al., 2008. Wilms' tumor gene *WT1*-shRNA as a potent apoptosis-inducing agent for solid tumors. Int. J. Oncol. 32 (3), 701–711.
Tong, D., et al., 2017. Increased Eps15 homology domain 1 and *RAB11FIP3* expression regulate breast cancer progression via promoting epithelial growth factor receptor recycling. Tumor Biol. 39 (2) 1010428317691010.
Tong, X., et al., 2009. Decreased *TIP30* expression promotes tumor metastasis in lung cancer. Am. J. Pathol. 174 (5), 1931–1939.
Varca, L.M., 2012. Pesticide residues in surface waters of Pagsanjan-Lumban catchment of Laguna de Bay, Philippines. Agric. Water Manag. 106, 35–41.
Wang, J., et al., 2016. Cyclin-dependent kinase 2 promotes tumor proliferation and induces radio resistance in glioblastoma. Translational Oncology 9 (6), 548–556.
Wang, S., et al., 2016b. Development of a prosaposin-derived therapeutic cyclic peptide that targets ovarian cancer via the tumor microenvironment. Sci. Transl. Med. 8 (329), 329ra34.
Wang, Y., et al., 2017. *C1QBP* suppresses cell adhesion and metastasis of renal carcinoma cells. Sci. Rep. 7 (1), 999.
Windham, G.C., et al., 1998. Genetic monitoring of malathion-exposed agricultural workers. Am. J. Ind. Med. 33 (2), 164–174.
Xu, M., et al., 2008. Epidermal growth factor receptor pathway substrate 8 is overexpressed in human pituitary tumors: role in proliferation and survival. Endocrinology 150 (5), 2064–2071.
Yao, J., et al., 2010. MicroRNA-30d promotes tumor invasion and metastasis by targeting Galphai2 in hepatocellular carcinoma. Hepatology 51 (3), 846–856.
Yao, W., et al., 2014. Profilin-1 suppresses tumorigenicity in pancreatic cancer through regulation of the SIRT3-HIF1α axis. Mol. Cancer 13 (1), 187.
Yasui, W., et al., 2004. Search for new biomarkers of gastric cancer through serial analysis of gene expression and its clinical implications. Cancer Sci. 95 (5), 385–392 2004.
Ye, Z.H., et al., 2017. Diagnostic and prognostic roles of *IRAK1* in hepatocellular carcinoma tissues: an analysis of immunohistochemistry and RNA-sequencing data from the cancer genome atlas. OncoTargets Ther. 10, 1711.
Zhang, Z., et al., 2009. Overexpression of follicle-stimulating hormone receptor facilitates the development of ovarian epithelial cancer. Cancer Lett. 278 (1), 56–64.
Zhao, M., Zhao, Z., 2016. Concordance of copy number loss and down-regulation of tumor suppressor genes: a pan-cancer study. BMC Genomics 17 (7), 532.
Zheng, J., et al., 2016. Apoptosis-related protein-1 acts as a tumor suppressor in cholangiocarcinoma cells by inducing cell cycle arrest via downregulation of cyclin-dependent kinase subunits. Oncol. Rep. 35 (2), 809–816.
Zhu, M., et al., 2014. Decreased *TIP30* promotes Snail-mediated epithelial-mesenchymal transition and tumor-initiating properties in hepatocellular carcinoma. Oncogene 34 (11), 1420.