Article

Oncogenic role of miRNA by environmental exposure to plasticizers: a systematic review.

Margherita Ferrante¹, Antonio Cristaldi and Gea Oliveri Conti¹

Abstract: The environmental exposure of human in the daily and occupational activities to plasticizers may adversely affect human health, and thus represents a global issue. The altered expression of MicroRNAs (miRNAs) exerts an important pathogenic role linked also to the exposure to plasticizers. This systematic review summarizes the recent findings showing modified expression of miRNAs in cancer due to plasticizers exposures. Following Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) methodology, we performed a systematic review of the past ten years, focusing on the relationship between plasticizer exposures and expression of miRNAs cancer. Starting by 535 records, 17 articles were included. Results support the hypothesis that exposure to plasticizers cause changes or deregulation of a number of oncogenic miRNAs and showed that plasticizers interaction with several redundant miRNAs, such as let-7f, let-7g, miR-125b, miR-134, miR-146a, miR-22; miR-192, miR-222, miR-26a, miR-26b, miR-27b, miR-29e, miR-324, miR-335, miR-122, miR-23b, miR-200, miR-29a and miR-21, might induce deep alterations. These genotoxic and oncogenic responses can eventually lead to abnormal cell signaling pathways and metabolisms that participate in many overlapped cellular processes, and miRNA level changes can be a useful tool for the toxicological assessment of environmental pollutants, including plastic additives and plasticizers.

Keywords: Plasticizers; Cancer; microRNA; in vitro study; PRISMA.

1. Introduction

The continuous environmental exposure of human in the daily and occupational activities to different chemicals may adversely affect human health, and thus represents a global issue [1–11]. Especially in the last decade the ecological and epidemiological studies focused on Plastic presence and their additives presence in food and environment [6,12–14].

Plasticizers are added to plastics as additives to increase their flexibility, durability, and pliability. A large broad of molecules are used by polymer industry including phthalates, bisphenolates, flame retardants, metals, parabens, polychlorinated biphenyls, tributyltin, organophosphate esters, etc.

Phthalates, between the plasticizers, are the most widely used in polyvinyl chloride (PVC), polyethylene terephthalate (PET), polyvinyl acetate (PVA), and polyethylene (PE) plastics, and these phthalates can be found in toys, personal-care products, food packages, paints, pharmaceuticals and drugs, medical devices, catheters, blood transfusion devices, cosmetics, and PVC products for home furnishings such as PVC films for floors or household accessories [12,15,16].

The Bisphenol A (BPA), another plasticizer, is the major component of manufacture of epoxy and polycarbonate plastics and flame retardants. The uses of BPA include coatings for PVC water pipe walls, plastic bottles for water, and baby bottles, food packaging, receipts inks, cosmetics, plastic toys, etc... BPA has focused high attention from researchers of public health and governmental agencies due to its high widespread human exposure. Also, BPA exerts genotoxic and carcinogenic activities due to similarity of its chemical structure that resembles that of the diethylstilbestrol, an accredited human carcinogen in humans [17,18]. Several risks has been reported to the about plasticizers exposures ranging by reproductive and developmental toxicant, until to the cancerogenic effects [19,20].

Plasticizers, in fact, have been associated with hormone-sensitive cancers such as breast, prostate, endometrial, ovarian, testicular and thyroid cancers but also with non-hormonal sensitive cancers such as cervical and lung cancers, osteosarcoma and meningioma [21].
The altered expression of MicroRNAs (miRNAs) represent an epigenetic mechanism that exerts an important pathogenic role linked to the exposure to environmental pollutants with several pathological outcomes, including cancer promotion and development [22,23, 24]. miRNAs are very short RNA molecules, ranging by 19 to 25 nucleotides in size, that regulate post-transcriptional silencing of target genes. A single miRNA can target until hundreds of miRNAs and influence the expression of a large number of genes often involved in several important functional pathways [22]. miRNAs are differentially regulated in various types of cancer, including ovarian, liver, gastric, pancreatic, esophageal, colorectal, breast, and lung cancers [25].

An emerging hypothesis is of a supposed coordination between miRNA-mediated gene control and splicing events in gene regulatory networks [23]. Several studies have suggested that maturation of specific miRNAs may depend on splicing factors [26]. However, microRNA modification results in carcinogenesis only when coupled also to other molecular changes occur simultaneously such as the suppression of inhibition the expression of mutated oncogenes, the microRNA adducts formation, p53-microRNA interconnections, and alterations of Dicer function [22]. Due to the marked considerable stability of miRNAs, they are measurable both in blood and tissues, and are therefore suitable can be eligible as potential biomarkers for several non-communicable diseases including cancer [25].

This systematic review summarizes the recent findings showing aberrant expression of miRNAs in cancer due to plasticizers exposures. We further discuss the challenges in environmental-miRNA research because of this approach can be an important key for the knowledge of mechanism of cancer pathophysiology but also for the early screening and or cancer personalized medicines therapy.

2. Materials and Methods

A brief critical review on scientific papers of the last ten years selected using PubMed, Scopus, Web of Science databases was carried out. “Preferred Reporting Items for Systematic Reviews and Meta-Analysis” (PRISMA) methodology was applied to perform this study.

To assess the influence of plasticizers exposures on miRNAs expression in human, all original articles published from January 1st, 2010 to December 29th, 2020 were selected based on the following criteria:
- be a original articles,
- to report plasticizer or plasticizers exposure
- to report miRNAs analysis and identification,
- have a correct scientific methodology,
- have miRNAs identification for cancers of all several target organs.

We searched the databases for controlled randomized studies, cohort studies, case-control studies, and case reports and in vitro studies. Only the original articles written in English were collected for the PRISMA review.

We excluded papers that did not include original data such as: informative review, commentaries, editorials. Systematic review and meta-analysis were selected as not eligible, but their references are screened for recovery eligible studies missing in databases.

Two investigators (A.C. and G.O.C.) screened all citations for potentially eligible studies and extracted data independently. Disagreements were adjudicated by a third investigator (M.F.).

The research was conducted using as keywords the terms “Plasticizers and microRNA”, “Plasticizers and oncogenic microRNA”, “additives of plastic and miRNA cancer”, “endoctrine disrupting chemicals and miRNA cancer”, “BPA and miRNA cancer”, “di-n-butyl phthalate and miRNA cancer”, “DBP and miRNA cancer”, “monobutyl phthalate and miRNA cancer”, “MBP and miRNA cancer”, “Organophosphorus flame retardants and miRNA cancer”, “flame retardants and miRNA cancer”, “di(2-ethylhexyl) phthalate and miRNA cancer”, “DEHP and miRNA cancer”, “mono-(2-ethylhexyl) phthalate and miRNA cancer”, “MEHP and miRNA cancer”, “methylparaben and miRNA cancer”, “parabens and miRNA cancer”, “phthalates and miRNA cancer”, “environmental phenols and miRNA cancer”, “organophosphate esters and miRNA cancer”, “Tributyltin and miRNA cancer”, “PCBs and miRNAs cancer”, “butylbenzyl phthalate and miRNA cancer”, “PVC and miRNA cancer”.

We also used combinations of the keywords such as “Plasticizers and oncogenic effects”, “Plasticizers and microRNA changes”.

All eligible studies were valuated using a modified New-Castle Ottawa scale [4].

3. Results

3.1 Studies included in the review
Our initial search produced 477 potential references (Figure 1) from databases and 58 references by other sources. Starting by a total of 535 records, we have identified in a first phase 322 records through after the deletion of 213 duplicates. In addition, through the title and abstract evaluation, we have screened 57 records. Of these, 40 records were excluded due to the absence of some criteria of eligibility (miRNAs reporting or indications, evidences about exposure to plasticizers, cancer tissue or cells analysis reporting) as listed in Fig. 1. We removed 155 duplicate papers, and after a careful review of the abstracts and full texts of the remaining 57 manuscripts. After a deep evaluation we excluded 40 articles for lacks miRNA data, or not evidence of plasticizers exposure, or for uncorrected methodology, or not cancer related miRNA detection, or no cancers cells lines or tissues analyses.

We finally included 17 studies on the systematic review.

These studies used various approaches or study designs, but all focused on the effects of the exposure to plasticizers on outcomes defined as “oncogenic miRNA identification and their down or up-regulation description”.

3.2. Summary of findings

Figure 1 describes the findings of the collection and screening performed. Starting by 535 records, we have identified in a first phase 322 records through after the deletion of 213 duplicates. In addition, through the title and abstract evaluation, we have screened 57 records. Of these, 40 records were excluded due to the absence of some criteria of eligibility as listed in Fig. 1. Therefore, this systematic review was carried out using 17 eligible full text records.

In alphabetical order we have included the following studies: Buñay et al. 2017 [27], Chang et al. 2017 [28], Chou et al. 2017 [29], Chorley et al. 2020 [30]; Cui et al. 2019 [31], Duan et al. 2020 [32], Hui et al. 2018 [33], Li et al. 2014 [34], Meng et al. 2013 [35], Scarano et al. 2019 [36]; Tilghman et al. 2012 [37], Kim et al. 2015 [38], Wang et al. 2019 [39], Wu et al. 2018 [40], Yin et al. 2018 [41], Zhu et al. 2019 [42], Zota et al. 2020 [43].
All studies are in vitro cells studies, ranging by rat, mice to human cancer cell lines. Several plasticizers were studied through controlled in vitro exposures as reported in Tab. 1.

Human endometrial, hemangioma, acute myeloid leukemia, ovarian, breast, hepatocellular, oral squamous and prostate cancer cell lines were evaluated.

**Table 1.** Included studies and results

| Study               | In Vitro/Vivo | Plasticizer | miRNA         | Expression | Reference n. |
|---------------------|---------------|-------------|---------------|------------|--------------|
| Buñay et al. 2017   | adult mice    | Cocktail (DEHP, DBP, BBP, NP, OP) | miR20b-5p    | Down       | [27]         |
|                     |               |             | miR-1291      | Down       |              |
| Chang et al. 2017   | *AS52 CHO cells inoculated in mouse | MEHP        | miR-let-7a    | Down       | [28]         |
|                     |               |             | miR-125b-5p   | Down       |              |
|                     |               |             | miR-125a-3p   | Down       |              |
|                     |               |             | miR-27a-3p    | Down       |              |
|                     |               |             | miR-25-3p     | Down       |              |
|                     |               |             | miR-92a-3p    | Down       |              |
| Chou et al. 2017    | **RL95–2 cell line | BPA         | miR-107       | Up         | [29]         |
|                     |               |             | miR-203       | Up         |              |
|                     |               |             | miR-205       | Up         |              |
|                     |               |             | miR-103a      | Up         |              |
|                     |               |             | miR-200c      | Up         |              |
|                     |               |             | miR-141       | Up         |              |
|                     |               |             | miR-221       | Up         |              |
|                     |               |             | Let-7a-5p     | Up         |              |
|                     |               |             | miR-193b      | Up         |              |
|                     |               |             | miR-423       | Up         |              |
|                     |               |             | miR-513       | Down       |              |
|                     |               |             | miR-149       | Down       |              |
|                     |               |             | miR-765       | Down       |              |
| Chorley et al. 2020 | serum and liver tissue of mice | DEHP, BBP and DNOP | miR-182–5p(DEHP) | Up | [30] |
|                     |               |             | miR-378a–3p(DEHP) | Up |              |
|                     |               |             | miR-125a–5p   | Up         |              |
| Cui et al. 2019     | *HA HDEC, CRL-2586 OEMA | MEHP, DEHP, DCHP and BBP | miR-655(BBP) | Down       | [31]         |
|                     |               |             | NE            |            |              |
| Duan et al. 2020    | βAML U937, Raji, and HL-60 cell lines. | BBP | miR-15b-5p    | Down       | [32]         |
|                     |               |             | miR-182      | NE         |              |
| Authors          | Treatment Conditions                     | miRNA | Regulation | Reference |
|------------------|-----------------------------------------|-------|------------|-----------|
| Hui et al. 2018  | SKOV3 and A2780 cell lines              | miR-21-5p, miR-22-3p | Up         | [33]      |
| Li et al. 2014   | MCF-7 cell line                         | miR-19a, miR-19b     | Up         | [34]      |
| Meng et al. 2013 | BEL-7402 cells and MCF-7 cells          | miR-21 | Down/a/Up b| [35]      |
| Scarano et al. 2019 | Pregnant rat exposure/ Ventral prostate tissues from puppies | miR-30d-5p | Up | [36] |
|                   | Cocktail DEHP, DEP, DBP, DiBP, BBzP, DiNP | miR-30b-5p, miR-141-3p, miR-30d-3p, mir-184 | Up | |
|                   |                                         | miR-342-3p | Down | |
|                   |                                         | miR-638   | Up    | |
|                   |                                         | miR-663   | Up    | |
|                   |                                         | miR-1915  | Up    | |
|                   |                                         | miR-93    | Up    | |
|                   |                                         | miR-320a  | Up    | |
|                   |                                         | miR-1,308 | Up    | |
|                   |                                         | miR-1,275 | Up    | |
|                   |                                         | miR-222   | Up    | |
|                   |                                         | miR-149   | Up    | |
|                   |                                         | miR-21    | Up    | |
| Tilghman et al. 2012 | MCF-7 cell line          | miR-21 | Down      | [37] |
|                   |                                        | let-7g    | Down    | |
|                   |                                        | let-7c    | Down    | |
|                   |                                        | miR-923   | Down    | |
|                   |                                        | let-7f    | Down    | |
|                   |                                        | miR-15b   | Down    | |
|                   |                                        | miR-27b   | Down    | |
|                   |                                        | miR-26b   | Down    | |
|                   |                                        | miR-342-3p| Down    | |
|                   |                                        | miR-638   | Up      | |
|                   |                                        | miR-663   | Up      | |
|                   |                                        | miR-1915  | Up      | |
|                   |                                        | miR-93    | Up      | |
|                   |                                        | miR-320a  | Up      | |
|                   |                                        | miR-1,308 | Up      | |
|                   |                                        | miR-1,275 | Up      | |
|                   |                                        | miR-222   | Up      | |
|                   |                                        | miR-149   | Up      | |
|                   |                                        | miR-21    | Up      | |
| Kim et al. 2015  | HepG2 cell line                        | miR-22   | Up        | [38] |
| Wang et al. 2019 | OSCC cells/subcutaneously injected in mice | miR-27b-5p | Down      | [39] |
| Wu et al. 2018   | MCF-7-MDA-MB-231                       | miR-19a, miR-19b | Up | [40] |
| Yin et al. 2018  | juvenile rat Sertoli cells              | miR-199a-3p | Up | [41] |
| miR-301b-3p | Up |
|miR-3584-5p  | Up |

**Zhu et al. 2019**

| miR-34a | Down | [42] |

**Zota et al. 2020** (FORGE study)

| miR-10a-5p | Up | [43] |

| miR-135a-5p | Up |
|miR-135b-5p  | Up |
|miR-137-3p   | Up |
|miR-302b-3p  | Up |
|miR-335-3p   | Up |
|miR-34a-5p   | Up |
|miR-34b-5p   | Up |
|miR-34c-5p   | Up |
|miR-483-5p   | Up |
|miR-488-3p   | Up |
|miR-488-5p   | Up |
|miR-508-3p   | Up |
|miR-577      | Up |
|miR-592      | Up |
|miR-651-5p   | Up |
|miR-885-5p   | Up |
|miR-9-3p     | Up |

* AS52-mutant cell (ASMC) clones; ** Human endometrial cancer cell line; † Human Hemangiomia cells; ‡ Acute myeloid leukemia; § Human ovarian cancer cell lines; †† Human breast cancer cells; †ERα-negative and estrogen-resistant); NE: no effect; UP: up regulated, Down: down regulated, ° Human hepatocellular carcinoma; ‡ Human oral squamous cell carcinoma; †† Human prostate cancer cells; ††† cancer cells; (10⁻⁴ or 10⁻⁵ M); (10⁻⁶ to 10⁻¹¹ M); Mono-ethylhexyl phthalate (MEHP); Bis (2-ethylhexyl) phthalate (DEHP); diethyl phthalate (DEP); dibutyl phthalate (DBP); di-isobutyl-phthalate (DiBP), butylbenzyl-phthalate (BBzP); di-isonylnyl-phthalate (DiNP); benzyl butyl phthalate (BBP); 4-nonylphenol (NP); 4-tert-octylphenol (OP); di-nonyl phthalate (DNOP); Tris (1,3-dichloro-2-propyl) phosphate (TDCIPP) = organophosphate flame retardants. Observational Research on Genes and the Environment (FORGE) study; ΣDEHP = Sum of 21 phthalates and metabolites; ΣAA phthalates = Sum of 31 antiandrogenic phthalate metabolites.
4. Discussion

Buñay et al. (2017) [27], have studied the consequences of the chronic exposure to a mixture of phthalates and alkylphenols on the testes of male mice and in particular reported the changes in the expression pattern of miRNA/iso-miRs which act as regulators of gene expression in testes. Also, the damage of testis and changes in the genes responsible for encoding proteins that are involved in the biogenesis, processing, editing, stability, or degradation of miRNAs were assessed. Buñay et al. study was carried out a case-control exposure study to mix of phthalates and alkylphenols using adult male mice exposed to a compared to control mice.

The E-exposed mice showed a degeneration of seminiferous tubules and hypertrophy/hyperplasia in specific parts of Leydig cells and also an increase of exfoliation of germ cells of seminiferous tubules that close the lumen or showed full closed tubules.

About mRNA levels, authors report that miRNAs of Star and Cyp17a1 and, Sp1 and Cyp11a1 were up-regulated and down-regulated, respectively. Instead, no significant differences in Hsd3b1 mRNA expression were detected.

Authors quantified the miRNAs expression levels of genes encoding proteins that are involved in pri-miRNAs processing (Drosha), nuclear export (Xpo5), stability/degradation (Lin28, Zcchc11, Zcchc6, and Snd1), editing (Adar) and processing of pre-miRNAs (Dicer, Ago2).

A significant increase of mRNA levels of Drosha, Adar, Zcchc11 in testes of exposed mice were found than those in control mice, contrary to Zcchc6, Dicer, Xpo5, Ago2, Lin28b, and Snd1 that showed no differences. Instead, mRNA levels of Zcchc6, Dicer, Xpo5, Ago2, Lin28b, and Snd1 no showed differences than to those of control mice.

miR20b-5p, miR-1291, that are implicated in cancer, and miR-3085-3p implicated in inflammation were found all down-regulated. miR-1291 targets DNA methyltransferases (Dnmt3a, Dnmt3b) that are involved in (de novo) histone methylation, genomic imprinting, X-chromosome inactivation and testicular germ cell tumors due to exposure to alkylphenols. In addition, Ccnd2, Ccnd1, and Raf1 are targets of the down-regulated miR-15b-5p in exposed mice and these targets are implicated in cell cycle regulation and in cancer. Hence, this study suggests that the downregulation of snRNAs through miR-1291 related to exposure to a plasticizers-mixture might promote changes in the DNA methylation pattern causing the epigenetic transmission of several diseases including cancer.

The mono-ethylhexyl phthalate (MEHP) is a metabolite of DEPH. The toxicity of MEHP is more potent than those of DEPH. Its toxic effects is more potent than those of the preprogenitor.

In Chang et al. study (2017) [28] the role of MEHP-induced reactive oxygen species (ROS) for genotoxicity was explained. Chang study provided the evidence for the of carcinogenicity of MEHP metabolite in Chinese hamster ovary AA8, UV5 and EM9 cells, as well as presenting insight into its capability to induce epigenetic modification for MEHP induction.

The cell lines were exposed to 0, 10, 25 and 50 mM MEHP, however at 50 mM MEHP all the cells died. The protection was not significant at 25 mM MEHP and, even after exposure to a lower dose of MEHP (1 mM), the PARP-1-KD cells have a higher level of single-strand breaks. The subsequently gpt gene sequencing to analyze the mutation points on the genesin AS52 mutant cells (ASMC) showed that 90% of all mutations were single base pair substitutions, especially G:C to A:T mutations. Independent AS52-mutant cell clones were collected for the performing of sequential in vivo xenograft tumorigenic studies, and 4 between 20 clones had aggressive tumor growth. The study showed also that miR-let-7a and miR-125b has been down-regulated in ASMC, which might raise oncogenic MYC and RAS level and might promote the activation of ErbB pathway. The mutagenic pathway of MEHP probably can be started through generation of ROS, causing base excision damage and so, resulting in carcinogenicity.

Chou et al. study (2017) [29] investigated the role of BPA exposure in disruption of miRNA regulation and if the related gene expression is decisive for carcinogenic progression. This study was carried out using human endometrial cancer RL95-2 cells with treatment of low to moderate BPA concentrations (10, 10² and 10³ nM).

Chou and colleagues reported that BPA exposure reduced the miR-149 expression to down-regulate DNA repair gene ARF6 (ADP ribosylation factor 6), and tumor protein p53 (TP53), and to up-regulate CCNE2 (cyclin E2). The results of the study showed also that BPA was also able to increase miR-107 to suppress hedgehog signaling factors, suppressor of fused homolog (SUFU) and GLI family zinc finger 3 (GLI3), providing a proof of potential epigenetic mechanism of BPA exposure on the endometrial carcinogenesis risk. In fact, miR107, miR149, miR200c, miR203, miR205, miR765 changed the genes (TP53, JUN, LAMB4, CCCDC6, PRKCA, STAT1, SUFI, CXCL8, DVL1, GLI3, CRK, LAMC1, MAPK1, MAPK9) of involved in cancer pathway (TP53, JUN, LAMB4, CCCDC6, PRKCA, STAT1, SUFI, CXCL8, DVL1, GLI3, CRK, LAMC1, MAPK1, MAPK9) with recording a significant fold change N>2.0 compared with control.
This study permitted the discovery and identification of five relevant pathways for the potential BPA-induced endometrial cancer progressions including cancer pathway, hedgehog pathway, cell cycle, adherens junction, and MAPK signaling pathway. In addition, TP53, GLI3, CCNE2, CRK, KIF23, SAMD2, CCD6, FZD3, ARF6, MAPK9, SUFI1, PRC1, MDM2, SMAD4, DVL1, EGLN1, JUN, MYC, LAMCI, PRKACA, and STAT1 were target significant differently expressed genes overlapped in these 5 pathways.

Chorley et al. (2020) [30] with their study measured liver and blood miRNAs in male B6C3F1 mice exposed both to a known chemical activator of the peroxisome proliferator-activated receptor alpha (PPARα) pathway perturbation and DEHP, respectively for 7 and 28 days at concentrations of 0, 750, 1500, 3000, or 6000 ppm through the oral exposure (feed). The PPARα pathway is a common target of several environmental chemicals. At the highest DEHP dose tested, 61 miRNAs were altered after 7 days and 171 miRNAs after 28 days of exposure, with 48 overlapping miRNAs between time points. Analysis of the 48 common miRNAs indicated the enrichment in PPARα–related targets and other pathways related to liver injury and cancer. The experiment was repeated using mmu-miRs-182-5p and -378a-3p analysis for DEHP, and di-n-octyl phthalate (DNOP) and n-butyl benzyl phthalate (BBP), other two related phthalates with weaker PPARα activity.

Results showed the greatest deregulatory potency of DEHP than DNOP and BBP, mmu-miRs-125a-5p, -182-5p, -20a-5p, and -378a-3p showed a clear dose relation, linked to the PPARα pathway. These findings identify also the putative miRNA biomarkers use of stratify chemical potency of plasticizers and generally, environmental pollutants.

Cui et al. (2019) [31], carried out a study to evaluate the potential roles of MEHP, DEHP, DCHP and BBP on Hemangioma progression, the most common tumors of infancy. This in vitro study was carried out using hemangioma cells, and authors have found that 100 nM BBP can significantly trigger the migration and invasion of hemangioma cells, inducing also the over-expression of Zeb1, a powerful transcription factor for cell migration and invasion, through miR-655 suppression or down regulation. Considering that 100 nM of BBP might also be measured in human tissues, the potential health risks of BBP on human, particularly for oncologic HA patients, should be paid more attention.

Duan et al. (2020) [32], also studied BBP effects on human acute monocytic leukemia AML U937 (isolated from the histiocytic lymph), Raji (lymphoblast Burkitt’s lymph), and HL-60 (a promyelocytic cell line) cell lines, and normal blood cells.

Doses of 10^−9 and 10^−4M of BBP were used for investigating the potential effect of BBP on malignancy of AML cells, instead for chemotherapy using a double exposure with increasing concentration of daunorubicin or cytarabine with or without 10^−4M BBP.

Results revealed that (10^−4M) BBP dosage can significantly decrease the sensitivity of daunorubicin and cytarabine. So, authors suggested that BBP can induce proliferation and reduce chemotherapy sensitivity of acute monocytic leukemia cells. PDK1, PDK2, PDK3, PDK4, PDP2, PDPR genes can regulate the glucose metabolism and glycolysis of cancer cells. In fact, cancer cells are characterized by high rates of glycolysis. The pyruvate dehydrogenase kinase (PDK) supports these energetic needs and also favors the apoptosis resistance. Duan showed that BBP increased the expression of PDK4 and PDP2 in U937 cells, while in Raji cells, BBP only increased the expression of PDK4. This study confirmed that BBP can decrease the expression of miR-15b-5p, while had no effect on miR-182, in both U937 and Raji cells. Over expression of miR-15b-5p can abolish BBP induced mRNA and protein expression of PDK4 in U937 cells. Further, the inhibitor of miR-15b-5p can increase the mRNA and protein expression of PDK4 in U937 cells.

Duan’s results suggested that down regulation of miR-15b-5p was involved in BBP induced PDK and demonstrated that BBP can increase the mRNA stability of PDK4 via down regulation of miR-15b-5p. Hence, BBP had no effect on the transcription and protein stability of PDK4, however, it significantly increased the mRNA stability of PDK4.

Hui et al. study (2018) [33] focused on BPA and ovarian cancer. The study was performed using an in vitro exposure to BPA (10 or 100 nM) or 0.1% DMSO for 24 hours using human ovarian adenocarcinoma SKOV3 cells, and then global gene expression profile was determined by high-throughput RNA sequencing. Transcriptional analysis revealed 94 differentially expressed genes related to tumorigenesis and metastasis. Authors revealed the up regulation of miR-21-5p and miR-222-3p, also they reported that BPA (10 and 100 nM) increased migration and invasion as well as induced epithelial to mesenchymal transitions in SKOV3 and A2780 cells. Accordingly, environmentally relevant-dose BBP has capability to activate the regular Wnt signaling pathway. This study first comprehensively analyzed the possible mechanisms underlying the effects of BPA on ovarian cancer. Environmentally relevant doses of BPA modulated the gene expression profile, promoted epithelial to mesenchymal transition progress via canonical Wnt signaling pathway of ovarian cancer.

Li et al. (2014) [34] studied how microRNAs are involved in curcumin-mediated protection from BPA-associated induced effects on breast cancer MCF-7 cell line. MCF-7 cell line was exposed to 10^−3M of BPA for 4 days. Results showed...
that BPA exhibited estrogenic activity by increasing the proliferation of estrogen-receptor-positive MCF-7 human breast cancer cells and promoting transition of the cells from G1 to S phase. Curcumin was capable to inhibit the proliferative effects of BPA on MCF-7 cells. In addition, BPA-induced up regulation of oncogenic miR-19a and miR-19b, and the dysregulated expression of miR-19-related downstream proteins, including PTEN, p-AKT, p-MDM2, p53, and proliferating cell nuclear antigen, were well reversed by curcumin. Furthermore, Li and colleagues highlighted the important role of miR-19 in BPA-mediated MCF-7 cell proliferation suggesting for the first time that curcumin modulates miR-19/PTEN/AKT/p53 axis to exhibit its protective effects against BPA-associated breast cancer.

Meng et al. (2013) [35] developed a miRNA biosensor and applied this novel tool to detect miRNA-21 extracted from human hepatocarcinoma BEL-7402 cells and human mastocarcinoma MCF-7 cells and their expression to BPA in vitro exposure. Normal human hepatic L-02 cells, BEL-7402 cells and MCF-7 cells were incubated with 100 μM BPA at same concentration for three days and five days, respectively. The expression profile of miRNA-21 in BEL-7402 and MCF-7 changed of 1.415-fold and 1.468-fold higher than that of normal L-02 cells, respectively showing that the miRNA expression levels of cancer cells were up regulated compared to those of normal cells.

Scarano et al. (2019) [36] studied the genome-wide levels of miRNAs to determine if perinatal exposure to a phthalate mixture in pregnant rats is capable to modify the gene expression during prostate development of the filial generation. The study was aimed to determine the epigenetic role of these pollutant in prostate cancer. Pregnant female Sprague Dawley rats were daily exposed (from gestational day 10 to postnatal day 21) to a mixture of phthalate by gavage and after were suppressed. Four groups were established: a control group exposed only to corn oil; (T1) 20 mg of the mixture (20 mg/kg/day); (T2) 200 mg of the mixture (200 mg/kg/day); and (T3) 200mg of the mixture (200 mg/kg/day). The cocktail contained DEHP, DEP, DBP, DiBP, BBzP and DiNP diluted in tocopherol-stripped corn oil. The 2 lower doses mimicked the daily human exposure levels based on the amount of DEHP, and the higher dose was selected to compare our results with available similar single previous phthalate studies. Rats from groups T1 to T3 received the respective doses of the phthalate’s cocktail prepared with 21% DEHP, 35% DEP, 15% DBP, 8% DiBP, 5% BBzP and 15% DiNP. After birth, the number of F1 offspring per litter was reduced to 8 (1: 1 ratio between males and females whenever possible), and litters with fewer than 6 pups were suppressed.

miRNAs in Treated Groups Versus Control were up regulated in T1 vs. C and in T2 vs. C. miR-141-3p was exclusively up regulated in the T1 vs. C group, whereas other miRNAs, such as miR-30d-5p, were deregulated in both groups with weak significant alteration in gene expression. miRNA-184 was up regulated in all treatment groups vs. C. Among possible targets for miR-141-3p (53 targets), 51 of them were down regulated. The MiRNAs differentially expressed in prostate tissue of these exposed animals were elicited in Tab.1. Scarano’s study, based on miRNAs evaluation, histopathological and immunostaining analysis, well support the hypothesis of the epigenetic role of phthalate in prostate oncogenesis.

Tilghman et al. (2012)[37] has studied the effects of BPA (10 μM) and DDT (10 μM) on miRNA regulation and expression levels in hormone-responsive human breast cancer cells. The MCF-7 breast cancer cell line exposure have showed that both the pollutants increased expression of ER receptor target genes, including progesterone receptor, bcl-2, and trefoil factor. The revealed miRNAs (27) were profiled outlined in exposed cells to 10 μM BPA and 10 μM DDT (miR-21, miR-638, miR-663, miR-1915, let-7g, let-7c, miR-923, miR-93, miR-320a, miR-1308, let-7f, miR-15b, miR-1275, miR-27b, miR-222, miR-193a-5p, miR-16, miR-26b, miR-149, miR-92a, miR-99b, miR-92b, miR-342-3p) of which several were up and down regulated according to the Table 1.

Several genes were differentially regulated by the compounds. For e.g. example, Jun and Fas genes were increased by approximately 1.8 and 1.5 fold by BPA but were relatively unchanged by DDT. The onco-miR-21 is an estrogen-regulated miRNA and plays an important role in breast cancer. In this study miR-21 expression was down regulated by BPA, and several members of the let-7 family (let-7a, let-7b, let-7c, let-7d, let-7e and let-7f), were down regulated (p<0.05) by all treatments. Instead miR-638 (P<0.005), miR-663 (P<0.005), and miR-1915 (P<0.005) were observed, instead after treatment of MCF7 cells with up regulated by BPA and DDT.

Kim et al. (2015) [38] study was performed using HepG2 cells that are widely used as a model system for studies of liver metabolism and genotoxicity. Especially authors determined the role of BPA-exposure in epigenetically affected expression of miR-22. Author have showed methylated Chr17:1565786-1565940 regions (promoter site for miR-22) in normal samples, but unmethylated in BPA-exposed samples. Kim et al. identified 7 differentially expressed miRNAs, including miR-22, in the BPA-exposed sample vs. control. Especially, in BPA-exposed samples, miR-22 showed 3.38 folds up-regulation than normal samples. Also, the Kim et al. study results support regulation of miR-22 expression by hypomethylation of the promoter region due to BPA-exposure.

Wang et al. (2019) [39], proposed their study aimed to evaluate the MEHP capability to promotes the proliferation of oral cancer through an in vitro/in vivo study using human oral squamous carcinoma (OSCC) (human OSCC-SCC-4, SCC-9, and SCC-25) cells and cell nuclear antigen (PCNA). SCC-4 cancer cells (2×106 per mouse) were diluted in 100 μl
normal medium and researcher injected these subcutaneously into the left flank of each mouse to obtain OSCC cancer xenografts. When the tumor grows to 100mm³, mice of MEHP's group were treated with MEHP (4 mg per kg, body weight) by intratumor injection for four times for every three days. The tumor volume was measured every 3 days and, at the end of the experiment, the mice were sacrificed and the xenograft tumors were removed to measure the expression of miRNAs and proteins.

Authors supported their hypothesis with solid results that showed proliferation of oral cancer by MEHP via down regulation of miR-27b-5p and miR-372-5p. In addition, MEHP induce the expression of c-Myc, which can suppress the transcription of miR-27b-5p in OSCC cells. So, the Wang’s study showed that MEHP can promote the growth and progression of OSCC via down regulation of miR-27b-5p and miR-372-5p.

Wu et al. (2018) [40] study showed that BBP induce proliferation of both ER(+) MCF-7 and ER(-) MDA-MB-231 breast cancer cells, proved by increased cell viability, transition of cell cycle from G1 to S phase, upregulation of PCNA and Cyclin D1, and downregulation of p21. Moreover, BBP modulated the expression of oncogenic miR-19a/b and PTEN/ AKT/p21 axis revealing that miR-19 plays a crucial role in the promoting effect of BBP on breast cancer cells through targeting PTEN 3’UTR. These findings provide an important tool for target intervention on cancer.

In Yin et al. (2018) [41] study, the global alterations of miRNA and mRNA expression in rat juvenile Sertoli cells (SCs) treated with 0.1 mM MBP were evaluated. Yin’s results revealed that miR-3584-5p and miR-301b-3p were up-regulated and their common target gene Dexamethasone-induced Ras-related protein 1 (Rasd1) was down-regulated. SCs proliferation induced by low MBP concentration in vitro could be mediated by Rasd1 regulating ERK1/2 signaling pathway. These results can represent a possible way to apply the personalized medicine screening and therapy in testicular tumors induced by exogenous chemicals.

Zhu et al. (2019) [42] study investigated the role of BBP in cell proliferation of prostate cancer cells. Human prostate cancer LNCaP and PC-3 cell lines were, in fact, exposed to low dose (0, 10⁻⁴, 10⁻⁵, 10⁻⁶, and 10⁻⁷ mol/L) of BBP for 6 days. Zhu’s results showed that 10⁻⁶ and 10⁻⁷ mol/L BBP increased the expression of cyclinD1 and PCNA, decreased p21 expression, and induced cell growth in both LNCaP and PC-3 cells vs. control group. Furthermore, authors found that BBP significantly downregulated the expression of miR-34a, along with upregulation of miR-34a target gene c-Myc. Using cell transfection of miR-34a mimic and inhibitor, so, authors demonstrated that in prostate cancer cells the trigger of BBP promoted cell proliferation that is mediated through the miR-34a/c-myc axis.

Zota et al. (2020) [43] represents the only human study included in this systematic review. The Fibroids, Observational Research on Genes and the Environment (FORGE) study involved 45 women living in Washington DC, during 2014-2017. Eligible women were nonpregnant, pre-menopausal, English-speaking, and ≥18 years of age. Authors quantified the expression levels of 754 miRNAs in fibroid tumor samples and analyzed the spot urine samples for phthalate metabolites collected from 45 pre-menopausal women undergoing hospital surgery for fibroid treatment.

Associations between miRNA levels in fibroids and phthalate biomarkers were evaluated using also a linear regression adjusted for age, race/ethnicity, and body mass index (BMI) and all statistical tests were adjusted also for multiple comparisons.

Spot urine samples from participants was collected and 1 urine sample was collected by a participant after surgery. Fibroid tissues, instead, were collected during hysterectomy or myomectomy procedures. For patients with multiple fibroids, only the largest fibroid was sampled.

In addition, the evaluation of single metabolites was carried out (Diethyl phthalate (DEP), Monoethyl phthalate (MEP), Di-n-butyl phthalate (DnBP), Mono-n-butyl phthalate (MnBP), Mono-hydroxybutyl phthalate (MHBP), Diisobutyl phthalate (DiBP), Monoisobutyl phthalate (MiBP), Mono-hydroxyisobutyl phthalate (MHiBP), Butylbenzyl phthalate (BBzP), Monobenzyl phthalate (MBzP), DnOP, Mono(3-carboxypropyl) phthalate (MCPP), Diisononyl phthalate (DiNP), Mono(2-carboxyphenoxy) phthalate (MCOP), Diisodecyl phthalate (DiDP), Mono(2-carboxyphenoxy) phthalate (MCNP), Di(2-ethylhexyl) phthalate (DEHP), Mono(2-ethylhexyl) phthalate (MEHP), Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHP), Mono(2-ethyl-5-oxohexyl) phthalate (MOEHP), Mono(2-ethyl-5-carboxypentyl) phthalate (MECPP), and authors also calculated 2 summary measures such as the molar sum of DEHP metabolites (ΣDEHP)21 and a potency-weighted sum of antidiandrogenic phthalate metabolites (ΣAA phthalates).

Fibroid characteristics was not different for race/ethnicity. Phthalate exposures were ubiquitous in enrolled woman; but 9 phthalate metabolites were detected in >90% of participants, however MEP levels were significantly higher in Black women. The enrolled women group were composed by Black (62%), overweight or obese (76%), privately insured (64%), and undergoing a myomectomy (58%). Compared with White/Latina women, Black women were more likely frequently to be obese, publicly insured, and having often a hysterectomy. miRNAs profiles detected were surprising for the social determinants’ aspects.

35 miRNAs were under-expressed, and 39 miRNAs were over-expressed in fibroid than myometrium.
Also, expression of: miR-10a-5p, miR-10a-3p, miR-140-3p, miR-144-5p, miR-150-5p, miR-205-5p, miR-27a-5p, miR-29b-2-5p, miR-29c-5p, miR-451a, and miR-95-3p were 3 fold greater in myometrium, instead expressions of miR-135a-5p, miR-135b-5p, miR-137-3p, miR-302b-3p, miR-335-3p, miR-34a-5p, miR-34a-3p, miR-34b-5p, miR-34c-5p, miR-483-5p, miR-488-3p, miR-488-5p, miR-508-3p, miR-577, miR-592, miR-651-5p, miR-885-5p, and miR-9-3p these miRNAs were 3 fold greater in fibroid.

Author found 285 significant associations between phthalate biomarkers and miRNAs (P <0.05), 34 of which were significant at P < 0.005.

After adjusting for multiple testing, we found 2 miRNAs associated with phthalate biomarkers, MHBp with increase in miR-10a-5p, 0.76 (95% CI = [0.40, 1.11]) and MEHHP with miR-577 (β= 1.06, 95% CI = [0.53, 1.59]).

8 phthalate-miRNA associations varied significantly between White/Latina and Black women and between these last there was an association between MBzP and miR-494-3p. Instead among white/Latina women, there were associations between MCP and miR-337-5p; MBzP and miR-1227-3p; MEP and miR-645; MEP and miR-564; MEP and miR-374-5p; MEHPP and miR-128-3p; and MEHP and miR-337-3p. 10 miRNAs that were significantly associated with phthalate biomarkers either in main analysis or in racial groups (miR-10a-5p, miR-577 miR-494-3p, miR- 377-5p, miR-1227-3p, miR-645, miR-564, miR-374a-5p, miR-128-3p, miR-337-5p).

Zota et al. have identified 923 mRNA targets that were experimentally observed or highly predicted targets of the 10 miRNAs, but 3 miRNAs (miR-10a-5p, miR-128-3p, miR-494-3p) were significantly associated with multiple fibroid-related processes including angiogenesis, apoptosis, proliferation of connective tissues, and cell viability, tumorigenesis of the reproductive tract, smooth muscle tumors.

miR-10a, miR-150, miR-29b, miR-29c, and miR-451 were under-expressed and miR-34a was over-expressed in fibroids. In particular the authors report that miR-10a-5p expression is associated with concentrations of MHBP, an oxidative metabolite of DnBP, which is found in some personal care products demonstrating that epigenome is sensitive to interactions between chemical and non-chemical stressors, but also to social determinants that can influence a wide range of physical and social environmental exposures altering the biological response to environmental pollutants.

5. Discussion and Conclusions

The epigenetic effects on DNA methylation including expression of miRNAs as effects of environmental chemicals such as plasticizers, including BPA and phthalates, have enlarged our knowledge about etiology of human chronic diseases such as cancer.

Several evidences, both from in vitro and in vivo models, have proved that epigenetic modifications due to exposure to common environmental pollutants can induce alterations in gene expression that may persist throughout life, exerting also a susceptibility to cancer risk. Epigenetics can affect the gene expression profiles of various organs and tissues.

The BPA and DEHP, MEHP, DBP, BBP and MBP between the phthalates, were found to make 1232 and 265 interactions with the same genes and proteins, respectively.

This systematic review shows that miRNA-based diagnostic models can predict several cancer organ’s targets in humans with high accuracy. Also, the evidences based about the carcinogenicity of several plasticizers were more supported by expression studies, permitting a future use of specific miRNA as valuable predictor or screening for early diagnosis biomarkers.

The miRNA level changes can be useful tools for the toxicological assessment of effects of several environmental pollutants, including plastic additives and plasticizers.

In this review we showed that plasticizers interaction with several redundant miRNAs such as let-7I, let-7g, miR-125b, miR-134, miR-146a, miR-22; miR-192, miR-222, miR-26a, miR-26b, miR-27b, miR-296, miR-324, miR-335, miR-122, miR-23b, miR-200, miR-29a and miR-21 might induce deep alterations of miRNA-mediated regulations and functions.

These genotoxic and oncogenic responses can eventually lead to abnormal cell signaling pathways and metabolisms that participate in many intercrossed or overlapped cellular processes.

BPA induces hypomethylation of histone promoter regions, indicating methylation changes as one of the possible mechanisms of BPA induced adverse effects on carcinogenesis. BPA is also involved in down regulation of: gene repair ARF6 (involved in cell differentiation, apoptosis and cell regulation), TP53 (a tumor suppressor gene also named as “Guardian of the Genome”), and over-regulate CCNE2 that is able to interact with CDKN1A and CDKN1B proteins, and with CDK3. The aberrant expression of CCNE2 is known to be a cause of cancer [44].
Phthalates have showed a down-regulation activity of some miRNAs (see Tab.1) implicated in cell cycle regulation and in cancer. Also, the activation and over-expression of ErbB, PPARα pathways, the generation of ROS, the over-expression of Zeb1 (transcription factor for cell migration and invasion) resulted by phthalates exposure studies.

It is important to highlight that the machinery by which plasticizers alter the epigenetic asset of cells are yet to be better elucidated in order to deeper understand the biology and biochemistry relatively to epigenetic alterations but also to disease-associated epigenetic alterations. The deeper knowledge of mechanisms will lead to better prediction of plasticizers health effects allowing to a more targeted, easy and appropriate disease prevention and therapy strategies.

The lack of human studies needs to be over-cross, the large quantities of experimental evidences will permit the proposal of dedicated epidemiological studies to evaluate the real effects of plasticizers on human health, especially for cancer derived by microplastics and their plasticizers that are too low studied and under valued by oncologists yet.

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References
1. Filippini, T., E. Heck, J., Malagoli, C., Del Giovane, C. & Vinceti, M. A Review and Meta-Analysis of Outdoor Air Pollution and Risk of Childhood Leukemia. *J. Environ. Sci. Health Part C Environ. Carcinog. Ecotoxicol. Rev.* 2015, 33, 36–66.
2. Oliveri Conti, G., Calogero, A. E., Giacone, F., Fiore, M., Barchitta, M., Agodi, A., Ferrante, M. B(a)P adduct levels and fertility: A cross-sectional study in a Sicilian population. *Mol. Med. Rep.* 2017, 15, 3398–3404.
3. Rapisarda, V., Loreto, C., Ledda, C., Musumeci, G., Bracci, M., Santarelli, L., Renis, M., Ferrante, M., & Cardile, V. Cytotoxicity, oxidative stress and genotoxicity induced by glass fibers on human alveolar epithelial cell line A549. *Toxicol. In Vitro* 2015, 29, 551–557.
4. Signorelli, S.S., Oliveri Conti, G., Zanobetti, A., Baccarelli, A., Fiore, M., Ferrante, M. Effect of particulate matter-bound metals exposure on prothrombotic biomarkers: A systematic review. *Environ. Res.* 2019, 177, 108573. https://doi.org/10.1016/j.envres.2019.108573.
5. De Domenico, E., Mauceri, A., Giordano, D., Maisano, M., Giofrè, G., Natalotto, A., D’Agata, A., Ferrante, M., Brundo, M. V., Fasulo, S. Effects of ‘in vivo’ exposure to toxic sediments on juveniles of sea bass (Dicentrarchus labrax). *Aquat. Toxicol. Amst. Neth.* 2011, 105, 688–697.
6. Cristaldi, A., Fiore, M., Zuccarello, P., Oliveri Conti, G., Grasso, A., Nicolosi, I., Copat, C., Ferrante, M. Efficiency of wastewater treatment plants (Wwtps) for microplastic removal: A systematic review. *Int. J. Environ. Res. Public. Health* 2020, 17, 1–23. https://doi.org/10.3390/ijerph17218014
7. Filippini, T., Tesauro, M., Fiore, M., Malagoli, C., Consonni, M., Violi, F., Iacuzio, L., Arcolin, E., Oliveri Conti, G., Cristaldi, A., Zuccarello, P., Zucchi, E., Mazzini, L., Pisano, F., Gagliardi, I., Patti, F., Mandrioli, J., Ferrante, M.,
8. Capolongo, S., Rebecchi, A., Dettori, M., Appolloni, L., Azara, A., Buffoli, M., Capasso, L., Casuccio, A., Oliveri Conti, G., D’Amico, A., Ferrante, M., Moscato, U., Oberti, I., Paglione, L., Restivo, V., D’Alessandro, D. Healthy Design and Urban Planning Strategies, Actions, and Policy to Achieve Salutogenic Cities. *Int. J. Environ. Res. Public. Health* **2018**, *15*(12), 2698. https://doi.org/10.3390/ijerph15122698.

9. Ferrante, M., Zanghì, G., Cristaldi, A., Copat, C., Grasso, A., Fiore, M., Signorelli, S. S., Zuccarello, P., & Oliveri Conti, G. PAHs in seafood from the Mediterranean Sea: An exposure risk assessment. *Food Chem. Toxicol.* **2018**, *115*, 385–390.

10. Conte, F., Copat, C., Longo, S., Conti, G. O., Grasso, A., Arena, G., Dimartino, A., Brundo, M. V., Ferrante, M Polycyclic aromatic hydrocarbons in Haliotis tuberculata (Linnaeus, 1758) (Mollusca, Gastropoda): Considerations on food safety and source investigation. *Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc.* **2016**, *94*, 57–63.

11. Zuccarello, P., Ferrante, M., Cristaldi, A., Copat, C., Grasso, A., Sanggregorio, D., Fiore, M., Oliveri Conti, G. Exposure to microplastics (<10 μm) associated to plastic bottles mineral water consumption: The first quantitative study. *Water Res.* **2019**, *157*, 365–371.

12. Oliveri Conti, G., Ferrante, M., Banni, M., Favara, C., Nicolosi, I., Cristaldi, A., Fiore, M., Zuccarello, P. Micro- and nano-plastics in edible fruit and vegetables. The first diet risks assessment for the general population. *Environ. Res.* **2020**, *187*, 109677.

13. Izzotti, A., Pulliero, A. The effects of environmental chemical carcinogens on the microRNA machinery. *Int. J. Hyg. Environ. Health* **2014**, *217*, 601–627.

14. Ferrante, M., Conti, G. O. Environment and Neurodegenerative Diseases: An Update on miRNA Role. *MicroRNA Shariqah United Arab Emir.* **2017**, *6*, 157–165.
24. Singh, S., Li, S.-L. Epigenetic Effects of Environmental Chemicals Bisphenol A and Phthalates. *Int. J. Mol. Sci.* **2012**, *13*, 10143–10153.

25. Asakura, K., Kadota, T., Matsuzaki, J., Yoshida, Y., Yamamoto, Y., Nakagawa, K., Takizawa, S., Aoki, Y., Nakamura, E., Miura, J., Sakamoto, H., Kato, K., Watanabe, S. I., Ochiya, T A miRNA-based diagnostic model predicts resectable lung cancer in humans with high accuracy. *Commun. Biol.* **2020**, *3*, 1–9.

26. Catalanotto, C., Cogoni, C. & Zardo, G. MicroRNA in Control of Gene Expression: An Overview of Nuclear Functions. *Int. J. Mol. Sci.* **2016**, *17*(10), 1712.

27. Bunay, J., Larriba, E., Moreno, R. D. & del Mazo, J. Chronic low-dose exposure to a mixture of environmental endocrine disruptors induces microRNAs/isomiRs deregulation in mouse concomitant with intratesticular estradiol reduction. *Sci. Rep.* **2017**, *7*(1):3373.

28. Chang, Y.-J., Tseng, C.-Y., Lin, P.-Y., Chuang, Y.-C. & Chao, M.-W. Acute exposure to DEHP metabolite, MEHP cause genotoxicity, mutagenesis and carcinogenicity in mammalian Chinese hamster ovary cells. *CARCINOGENESIS* **2017**, *38*, 336–345.

29. Chou, W. C., Lee, P. H., Tan, Y. Y., Lin, H. C., Yang, C. W., Chen, K. H., & Chuang, C. Y. An integrative transcriptomic analysis reveals bisphenol A exposure-induced dysregulation of microRNA expression in human endometrial cells. *Toxicol. In Vitro* **2017**, *41*, 133–142.

30. Chorley, B. N., Carswell, G. K., Nelson, G., Bhat, V. S. Wood, C. E. Early microRNA indicators of PPARα pathway activation in the liver. *Toxicol. Rep.* **2020**, *7*, 805–815.

31. Cui, S., Wang, L., Zhao, H., Lu, F., Wang, W., & Yuan, Z. Benzyl butyl phthalate (BBP) triggers the migration and invasion of hemangioma cells via upregulation of Zeb1. *Toxicol. In Vitro* **2019**, *60*, 323–329.

32. Duan, X.-L., Ma, C.-C., Hua, J., Xiao, T.-W. Luan, J. Benzyl butyl phthalate (BBP) triggers the malignancy of acute myeloid leukemia cells via upregulation of PDK4. *Toxicol. In Vitro* **2020**, *62*, 104693.

33. Hui, L., Li, H., Lu, G., Chen, Z., Sun, W., Shi, Y., Fu, Z., Huang, B., Zhu, X., Lu, W., Xia, D., Wu, Y. Low Dose of Bisphenol A Modulates Ovarian Cancer Gene Expression Profile and Promotes Epithelial to Mesenchymal Transition Via Canonical Wnt Pathway. *Toxical Sci. Off. J. Soc. Toxicol.* **2018**, *164*, 527–538.

34. Li, X., Xie, W., Xie, C., Huang, C., Zhu, J., Liang, Z., Deng, F., Zhu, M., Zhu, W., Wu, R., Wu, J., Geng, S., Zhong, C. Curcumin modulates miR-19/PTEN/AKT/p53 axis to suppress bisphenol A-induced MCF-7 breast cancer cell proliferation. *Phytother. Res.* **2014**, *28*, 1553–1560.

35. Meng, X., Zhou, Y., Liang, Q., Qu, X., Yang, Q., Yin, H., Ai, S. Electrochemical determination of microRNA-21 based on bio bar code and hemin/G-quadruplet DNAenzyme. *Analyst* **2013**, *138*(12), 3409–3415.

36. Scarano, W. R., Bedrat, A., Alonso-Costa, L. G., Aquino, A. M., Fantinatti, B., Justulin, L. A., Barbisan, L. F., Freire, P. P., Flaws, J. A., Bernardo, L. Exposure to an environmentally relevant phthalate mixture during prostate development induces microRNA upregulation and transcriptome modulation in rats. *Toxical Sci. Off. J. Soc. Toxicol.* **2019**, *171*(1), 84–97. doi:10.1093/toxsci/kfz141.

37. Tilghman, S. L., Bratton, M. R., Segar, H. C., Martin, E. C., Rhodes, L. V., Li, M., McLachlan, J. A., Wiese, T. E., Nephew, K. P., Burow, M. Endocrine Disruptor Regulation of MicroRNA Expression in Breast Carcinoma Cells. *PLOS ONE* **2012**, *7*, e32754.

38. Kim, S. J. *et al.* Epigenetic Regulation of miR-22 in a BPA-exposed Human Hepatoma Cell. *BioChip J.* **9**, 76–84 (2015).

39. Wang, M., Qiu, Y., Zhang, R., Gao, L., Wang, X., Bi, L., Wang, Y. MEHP promotes the proliferation of oral cancer cells via down regulation of miR-27b-5p and miR-372-5p. *Toxicol. In Vitro* **2019**, *58*, 35–41.

40. Wu, J. *et al.* miR-19 targeting of PTEN mediates butyl benzyl phthalate-induced proliferation in both ER(+) and ER(-) breast cancer cells. *Toxicol. Lett.* **295**, 124–133 (2018).
41. Yin, X. et al. MiR-301b-3p/3584-5p enhances low-dose mono-n-butyl phthalate (MBP)–induced proliferation by targeting Rasd1 in Sertoli cells. *Toxicol. In Vitro* **47**, 79–88 (2018).

42. Zhu, M. et al. Butyl benzyl phthalate promotes prostate cancer cell proliferation through miR-34a downregulation. *Toxicol. In Vitro* **54**, 82–88 (2019).

43. Zota, A. R. et al. Phthalate Exposures and MicroRNA Expression in Uterine Fibroids: The FORGE Study. *Epigenetics Insights* **13**, 2516865720904057 (2020).

44. Kreike, B., Hart, G., Bartelink, H., & van de Vijver, M. J. Analysis of breast cancer related gene expression using natural splines and the Cox proportional hazard model to identify prognostic associations. *Breast cancer research and treatment*, 2010, **122**(3), 711–720.