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Article

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

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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-296, 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-hormone~~al~~ sensitive cancers such as cervical and lung cancers, osteosarcoma and meningioma [21].

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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 mRNAs 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~~ suggest 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", "endocrine 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", "Organophoshorus 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 microRNAs 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

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Our initial search produced 477 potential references (Figure 1) from databases and 58 references by other sources.

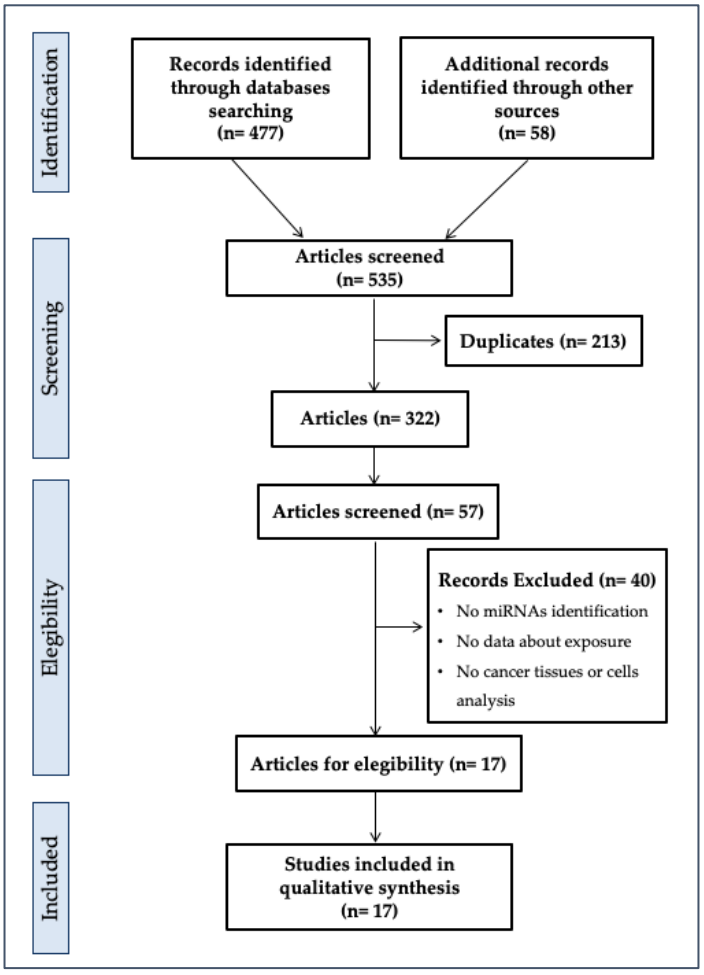
Starting by a total of 535 records, we have identified in a first phase 322 records ~~through~~ after the deletion of 213 duplicates. In addition, through the title and abstract evaluation, we have screened 57 records. Of these, 40 records were excluded due to the absence of some criteria of eligibility (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 S systematic review was carried out using 17 eligible full text records.~~



REVISED Figure 1. PRISMA Flow diagram

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].

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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 cells 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-130a-3p	Down	
			miR-27a-3p	Down	
			miR-25-3p	Down	
			miR-92a-3p	Down	
Chou et al. 2017	**RL95–2cell 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	$^{\beta}$AML U937, Raji, and HL-60 cell lines.	**BBP**	miR-15b-5p	Down	[32]
			miR-182	NE	

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Hui et al. 2018	§SKOV3 and §A2780 cell lines	**BPA**	miR-21-5p miR-222-3p	Up Up	[33]
Li et al. 2014	^MCF-7 cell line	**BPA**	miR-19a miR-19b	Up Up	[34]
Meng et al. 2013	$BEL-7402 cells ^MCF-7 cells	**BPA**	miR-21 miR-21	Down[a]/Up[b] Down[a]/Up[b]	[35]
Scarano et al. 2019	**Pregnant rat exposure/ Ventral prostate tissues from puppies**	**Cocktail DEHP, DEP, DBP, DiBP, BBzP, DiNP**	miR-30d-5p miR-30b-5p miR-141-3p miR-30d-3p mir-184	Up Up Up Up Up	[36]
Tilghman et al. 2012	^MCF-7 cell line	**BPA**	miR-21 let-7g let-7c miR-923 let-7f miR-15b miR-27b miR-26b miR-342-3p miR-638 miR-663 miR-1915 miR-93 miR-320a miR-1,308 miR-1,275 miR-222 miR-149	Down Down Down Down Down Down Down Down Down Up Up Up Up Up Up Up Up Up	[37]
	ϙMCF-7F cells		miR-21	Up	
Kim et al. 2015	°HepG2 cell line	**BPA**	miR-22	Up	[38]
Wang et al. 2019	ΣOSCC cells/ subcutaneously injected in mice	**MEHP**	miR-27b-5p miR-372-5p	Down Down	[39]
Wu et al. 2018	^MCF-7 ^MDA-MB-231	**BBP**	miR-19a miR-19b	Up Up	[40]
Yin et al. 2018	juvenile rat Sertoli cells		miR-199a-3p	Up	[41]

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			miR-301b-3p	Up	
			miR-3584-5p	Up	
Zhu et al. 2019	[π]LNCaP and PC-3cells	**BBP**	miR-34a	Down	[42]
Zota et al. 2020 (FORGE) study	Human Fibroid and myometrium tissue - Uterine Leiomyoma	**ΣDEHP and ΣAA phthalates**	miR-10a-5p	Up	[43]
	myometrium		miR-10a-3p	Up	
	myometrium		miR-140-3p	Up	
	myometrium		miR-144-5p	Up	
	myometrium		miR-150-5p	Up	
	myometrium		miR-205-5p	Up	
	myometrium		miR-27a-5p	Up	
	myometrium		miR-29b-2-5p	Up	
	myometrium		miR-29c-5p	Up	
	myometrium		miR-451a	Up	
	myometrium		miR-95-3p	Up	
	Fibroid		miR-135a-5p	Up	
	Fibroid		miR-135b-5p	Up	
	Fibroid		miR-137-3p	Up	
	Fibroid		miR-302b-3p	Up	
	Fibroid		miR-335-3p	Up	
	Fibroid		miR-34a-5p	Up	
	Fibroid		miR-34a-3p	Up	
	Fibroid		miR-34b-5p	Up	
	Fibroid		miR-34c-5p	Up	
	Fibroid		miR-483-5p	Up	
	Fibroid		miR-488-3p	Up	
	Fibroid		miR-488-5p	Up	
	Fibroid		miR-508-3p	Up	
	Fibroid		miR-577	Up	
	Fibroid		miR-592	Up	
	Fibroid		miR-651-5p	Up	
	Fibroid		miR-885-5p	Up	
	Fibroid		miR-9-3p	Up	

*AS52-mutant cell (ASMC) clones; **Human endometrial cancer cell line; [+]Human Hemangioma cells; [β]Acute myeloid leukemia; [§]Human ovarian cancer cell lines; ^Human breast cancer cells; [ρ](ERα-negative and estrogen-resistant); NE: no effect; UP: up regulated, Down: down regulated, °Human hepatocellular carcinoma; [Σ] Human oral squamous cell carcinoma; [π]Human prostate cancer cells; [$]cancer cells; [a]($10^{-4}$ or 10^{-5} M); [b](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-isononyl-phthalate (DiNP); benzyl butyl phthalate (BBP); 4-nonylphenol (NP); 4-tert-octylphenol (OP); di-noctyl 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.

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4. ~~Discussion~~Results

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/isomiRs 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 mRNAs 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* ~~mRNA levels~~ 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 sncRNAs 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 progenitor,~~

In **Chang et al. study (2017)** [28] the role of MEHP-induced reactive oxygen species (ROS) for genotoxicity ~~were~~ 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, 10^3 and 10^5 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 ~~of~~. In fact, miR107, miR149, miR200c, miR203, miR205, miR765 changed the genes (*TP53, JUN, LAMB4, CCCDC6, PRKCA, STAT1, SUFU, CXCL8, DVL1, GLI3, CRK, LAMC1, MAPK1, MAPK9*) ~~of~~ involved in cancer pathway ~~(*TP53, JUN, LAMB4, CCCDC6, PRKCA, STAT1, SUFU, CXCL8, DVL1, GLI3, CRK, LAMC1, MAPK1, MAPK9*)~~ ~~with~~ recording a significant fold change N>2.0 compared with control.

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This study permitted the discovery and identification of five relevant pathways ~~relevant~~ 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*, *CCDC6*, *FZD3*, *ARF6*, *MAPK9*, *SUFU*, *PRC1*, *MDM2*, *SMAD4*, *DVL1*, *EGLN1*, *JUN*, *MYC*, *LAMC1*, *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 ~~in~~ 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^{-4}M of BBP were used for investigating the potential effect of BBP on malignancy of AML cells, instead for carrying out mechanistic study was used a dose of 10^{-8}M. Authors checked effects of BBP on the proliferation of AMLU937, Raji, and HL-60 cell lines. Further, authors verified the BBP perturbation on treated U937 cells against the efficacy of ~~chemiotherapic~~s chemotherapy using a double exposure with increasing concentration of daunorubicin or cytarabine with or without 10^{-8}M BBP.

Results revealed that (10^{-8}M) 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. Transcriptomic analysis revealed 94 differen~~tial~~ expression 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 BPA has capable 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-~~$^{-5}$M of~~ BPA for 4 days. Results showed

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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 mRNAs 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 promote~~s~~ 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

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normal medium and researcher injected these subcutaneously into the left flank of each mouse to obtain OSCC cancer xenografts. When the tumor grows to 100mm^3, mice of MEHPs group were treated with MEHP (4 mg per kg, body weight) by intratumorally 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},10^{-7} and 10^{-8} 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 ~~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), Monocarboxyoctyl phthalate (MCOP), Diisodecyl phthalate (DiDP), Monocarboxynonyl phthalate (MCNP), Di(2-ethylhexyl) phthalate (DEHP), Mono(2-ethylhexyl) phthalate (MEHP), Mono(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP), Mono(2-ethyl-5-oxohexyl) phthalate (MEOHP), 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 antiandrogenic 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.

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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-9-3pthese 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 MCPP and miR-337-5p; MBzP and miR-1227-3p; MEP and miR-645; MEP and miR-564; MEP and miR-374-5p; MEHP 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- 337-5p, miR-1227-3p, miR-645, miR-564, miR-374a-5p, miR-128-3p, miR-337-3p).

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 mirRNAs 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-7f, 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 valuated by oncologists yet.

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