Accepted Manuscript

Title: The expression of microRNAs and exposure to environmental contaminants related to human health

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Received 24 Jan 2020, Accepted 14 Apr 2020, Published online: 12 May 2020

https://doi.org/10.1080/09603123.2020.1757043

International Journal of Environmental Health Research

Please cite this article as: Maria Rosaria Tumolo, Alessandra Panico, Antonella De Donno, Pierpaolo Mincarone, Carlo Giacomo Leo, Roberto Guarino, Francesco Bagordo, Francesca Serio, Adele Idolo, Tiziana Grassi & Saverio Sabina (2022) The expression of microRNAs and exposure to environmental contaminants related to human health: a review, International Journal of Environmental Health Research, 32:2, 332-354, DOI: 10.1080/09603123.2020.1757043.

The expression of microRNAs and exposure to environmental contaminants related to human health: a review

Journal:	*International Journal Of Environmental Health Research*
Manuscript ID	CIJE-2020-0035.R1
Manuscript Type:	Review Article
Keywords:	microRNAs, pathway, environmental pollutants, health

The expression of microRNAs and exposure to environmental contaminants related to human health: a review

Abstract

Environmental contaminants exposure may lead to detrimental changes to the microRNAs (miRNAs) expression resulting in several health effects. miRNAs, small non-coding RNAs that regulate gene expression, have multiple transcript targets and thereby regulate several signaling molecules. Even a minor alteration in the abundance of one miRNA can have deep effects on global gene expression. Altered patterns of miRNAs can be responsible for changes linked to various health outcomes, suggesting that specific miRNAs are activated in pathophysiological processes. In this review, we provide an overview of studies investigating the impact of air pollution, organic chemicals, and heavy metals on miRNA expression and the potential biologic effects on humans.

Keywords: microRNAs, pathway, environmental pollutants, health

Abbreviations: AHRR, aryl-hydrocarbon receptor repressor; AHR, aryl-hydrocarbon receptor; As, arsenic; BCL2, B-cell lymphoma 2; BCL2L11, B-cell lymphoma 2 like 11; BCL6, B-cell lymphoma 6; BPA, bisphenol A; CVD, cardiovascular diseases; CD40, cluster of differentiation 40; CCND1, Cyclin D1; CDKN1A, cyclin-dependent kinase inhibitor 1A; ~~COL1A2, collagen type I alpha 2 chain~~; Cr, chromium; ~~CSF1, colony stimulating factor 1;~~ CTBP1, C-terminal binding protein 1; CXCL12, C-X-C motif chemokine ligand 12; ~~CYP3A4, cytochrome P450 family 3 subfamily A member 4; CYP2E1, cytochrome P450 family 2 subfamily E member ;~~ DAZAP1, deleted in azoospermia associated protein 1; DEP, diesel exhaust particles; EGFR, epidermal growth factor receptor; eNOS, endothelial nitric oxide synthase; ~~ERB1, eukaryotic ribosome biogenesis protein;~~ EVs, extracellular vesicles; FAK, focal adhesion kinase; FAS, fas cell surface death receptor; FOXO~~1~~, forkhead box O~~1~~; ~~GSTP1, glutathione-S-transferase pi 1;~~ HbA1c, glycated hemoglobin; Hg, mercury; HLA-A,

human leukocyte antigen A; HMGB~~1/AGER~~, high mobility group protein B ~~box 1/advanced glycosylation end-product specific receptor~~; ~~ICAM-1, intercellular adhesion molecule 1;~~ IFNAR2, interferon alpha receptor subunit 2; IL-6, interleukin-6; IRAK1, interleukin 1 receptor associated kinase 1; JAK/STAT, janus kinase/signal transducers and activators of transcription; ~~LRRK2, leucine-rich repeat kinase 2;~~ MAPK, mitogen-activated protein kinase; ~~MEF2C, myocyte enhancer factor 2C~~; miRNAs, microRNAs; MVs, microvesicles; NCDs, noncommunicable diseases; NFAT, nuclear factor of activated T cells; NFkB, nuclear factor kappa B; NRF2, nuclear factor, erythroid-derived 2; ~~NFE2L2, nuclear factor, erythroid 2 like 2 NGF, nerve growth factor;~~ NRG3, neuregulin 3; O_3, ozone; OP, organophosphorus pesticides; PAHs, polycyclic aromatic hydrocarbons; Pb, lead; PCBs, polychlorinated biphenyls; PDCD4, programmed cell death 4; PDGFB, platelet derived growth factor subunit beta; PDGFR, platelet derived growth factor receptor; PI3K/Akt, phosphoinositide-3-kinase/protein kinase B; PKA, protein kinase A; PM, particulate matter; PRKCQ, protein kinase C theta; PTEN, phosphatase and tensin homolog; ~~PTGES3, prostaglandin E synthase 3; SLAM-SAP, signaling lymphocytic activation molecule-associated protein;~~ SORT1, sortilin 1; TGFβ, transforming growth factor-β; ~~TFIIH, transcription factor II human;~~ TLR, toll-like receptor; TNF, tumor necrosis factors; TRAF16, tumor necrosis factors-receptor associated factors 16; TRAP, traffic-related air pollution; TREM1, triggering receptor expressed on myeloid cells 1; TRIAP1, TP53 regulated inhibitor of apoptosis 1; VCAM-1, vascular cell adhesion molecule 1; VEGFA, vascular endothelial growth factor A; XRCC2, X-ray repair cross complementing 2; YBX2, Y-box-binding protein 2; ZEB1, zinc finger E-box-binding homeobox 1; ZEB2, zinc finger E-box-binding homeobox 2; 8-OH-dG, 8-hydroxy-guanine.

Introduction

Exposure to environmental contaminants, including air pollution, organic chemicals, and heavy metals, is a global public health problem associated with adverse health effects (Humphrey et al. 2019). Some sub-cellular effects caused by environmental factors have been investigated both in vitro (Bonetta et al. 2019) and in vivo (Domingues et al. 2018; Panico et al. 2020). Recent evidence suggests that the exposure to toxic compounds influences microRNAs (miRNAs) expression, which contributes to disease development later in life (Miguel et al. 2018).

miRNAs are a class of short non-coding RNA with 18-25 nucleotides in length (Popovic et al. 2013) that play an active role in epigenetic regulation of gene expression, and are also involved in post-transcriptional gene silencing. They have been detected not only intracellularly, but also in extracellular human body fluids, such as serum/plasma, saliva, urine, etc. (Gallo et al. 2012). Despite is the presence of high extracellular RNase activity, miRNAs are highly stable in extracellular area since packaged in apoptotic bodies, microvesicles (MVs), or high density lipoprotein particles (Turchinovich et al. 2012). ~~Therefore,~~

miRNAs regulate many aspects of biology, including developmental timings, cell differentiation, intercellular communication, embryogenesis, metabolism, organogenesis, and apoptosis (Turchinovich et al. 2016). Through multiple transcript targets, miRNAs regulate signaling molecules or pathways, and they can even be transcriptional targets, providing a mechanism for dysregulation of genes by activation of transcription factors (Hoesel & Schmid 2013). Even a minor alteration to the abundance of one miRNA can have deep effects on global gene expression (Humphrey et al. 2019).

Altered patterns of miRNAs can be responsible for changes linked to various health outcomes, suggesting that specific miRNAs are activated in pathophysiological processes (Ardekani & Naeini 2010).

Recently, many investigations have examined the relationship between environmental factors and miRNAs expression, identifying several chemical contaminants that dysregulated this class of

molecules (Vrijens et al. 2015). As such, we need to understand the biological process underlying miRNA alteration in response to environmental contaminants in order to explore their potential as biomarkers in the management of noncommunicable diseases (NCDs) linked to environmental exposure. This narrative review provides an overview of studies in humans investigating the impact of environmental factors, that is air pollution, organic chemicals, and heavy metals, on miRNA expression, also considering the potential biological mechanism that may lead to pathological condition. ~~and the potential biologic effects on humans.~~

miRNAs affected by air pollution

Particulate Matter (PM). Particulate matter consists of a mixture of airborne particles originated from natural sources and anthropogenic activities. Data show that acute PM exposure leads to adverse health effects through oxidative stress generation and inflammation induction, with the highest impacts on cancer and cardiovascular diseases (CVD) (Martinelli et al. 2013).

Table 1 summarizes the studies on the expression of miRNAs in response to air pollution, also including sample types, study designs, and methodologies for miRNA detection and for analysis of their targets. miRNA nomenclature was reported adopting the most recent official version used in *miRbase* (Griffiths-Jones et al. 2008; Kozomara et al. 2019).

Insert Table 1 about here.

PM concentration has been linked to several clinical manifestations of CVD, which, in turn, lead to altered miRNAs expression (Vrijens et al. 2015). Louwies and colleagues conducted a study that investigated relationship between air pollutants and miRNAs expression in combination with microvascular responses to PM. They measured by real-time quantitative reverse-transcriptase polymerase chain reaction (qRT-PCR) the levels of three candidate miRNAs in the blood of 50 healthy adults. Of the three miRNAs examined, only miR-21-5p and miR-222-3p were negatively associated with PM_{10}. ~~A retinal microvascular response to variation in PM could be explained by dysregulated miRNAs, miR-21-5p and miR-222-3p, in blood of healthy adults.~~ Bioinformatic

analysis revealed gene targets of these miRNAs that were associated with ~~These miRNAs are involved in~~ inflammatory ~~and oxidative stress~~ pathways, that is phosphatase and tensin homolog (PTEN) signaling pathway and high-mobility group protein B (HMGB). They also assessed the width of retinal blood vessels by eye fundus photos. Thus, a retinal microvascular response to variation in PM could be explained by these dysregulated miRNAs, that have a role in these pathways (Louwies et al. 2016). ~~Their effect on the high mobility group box 1 (HMGB1)/advanced glycosylation end-product-specific receptor (AGER) signaling pathway leads to enhanced production of proinflammatory cytokines, adhesion molecules, and coagulation factors . Additionally, the down-regulation of these miRNAs in the phosphatase and tensin homolog (PTEN) signaling pathway upregulates PTEN expression that in turn either inhibits the endothelial nitric oxide synthase (eNOS) pathway or increases intercellular adhesion molecule 1 (ICAM-1) expression resulting in retinal vessels narrowing, or both (Louwies et al., 2016).~~

~~The same miRNAs were significantly increased in blood of steel plant workers collected after three workdays exposed to high PM. For these specific miRNAs, miR-222 is associated with mitogen-activated protein kinase (MAPK) signaling and nerve growth factor (NGF) signaling, while miR-21 is associated with PTEN, MAPK, and phosphoinositide-3-kinase (PI3K)/protein kinase B (Akt) signaling (Bollati et al. 2010). In other studies of PM exposure implicated in CVD, microvesicle-associated miRNA expression (miR-128 and miR-302c) was overexpressed after three days of workplace PM exposure in healthy electric steel plant facility workers. In this case study, nuclear factor kappa B (NFkB), commonly associated with a prototypical pro-inflammatory signaling pathway, was found to be a central molecule in the networks of both miRNAs (Bollati et al. 2015).~~

Air pollution could alter intercellular communication by extracellular vesicles (EVs), such as MVs, that can transfer miRNAs between tissues (Pavanello et al. 2016). Rodosthenous et al. investigated relationship between short-, intermediate-, and long-term exposures to PM and levels of EV-miRNAs in a cohort of healthy adults. The profile of 800 miRNAs was screened using Nanostring Technologies' nCounter® assay that revealed an association between long-term ambient PM exposure

~~is also associated~~ with increased ~~disrupting background~~ levels of some~~sixteen~~ EV-~~extracellular vesicle~~ ~~(EV)~~ miRNAs circulating in serum; in silico analysis showed that their ~~which~~ target genes (for example interleukin 6 - IL-6, C-X-C motif chemokine ligand 12 - CXCL12, vascular cell adhesion molecule 1 - VCAM-1, cluster of differentiation 40 - CD40, platelet derived growth factor subunit beta - PDGFB, etc.) are linked ~~relevant~~ to CVD-related pathways, such as ~~oxidative stress,~~ inflammatory response,~~ion and~~ atherosclerosis, toll-like receptor (TLR) etc. (Rodosthenous et al. 2016). ~~Among these miRNAs, miR-146a-5p is an important regulator of pro-inflammatory cytokines such as interleukin-6 (IL-6) via the NFkB pathway; moreover, this miRNA targets tumor necrosis factors (TNF) receptor-associated factors 6 (TRAF6) and interleukin 1 receptor-associated kinase 1 (IRAK1), proteins that are part of the cluster of differentiation 40 (CD40) signaling pathway, which serves an important role in cellular communication during inflammatory responses ; miR-23a-3p interacts with C-X-C motif chemokine ligand 12 (CXCL12), which has been reported to regulate inflammation; miR-126a-3p is associated with vascular cell adhesion molecule 1 (VCAM-1) expressed by endothelial cells in response to inflammation and plays a critical role in recruiting leukocytes with implications for vascular inflammation and atherosclerosis ; miR-150-5p interacts with platelet-derived growth factor subunit beta (PDGFB), a protein expressed by smooth muscle endothelial and epithelial cells that plays a central role in cell proliferation and has been implicated in inflammatory responses and atherosclerosis; let-7g and miR-130-3p target other cytokines, such as colony-stimulating factor 1 (CSF1) and collagen type I alpha 2 chain (COL1A2), mediating communication between immune cells in the inflammatory process (Rodosthenous et al. 2016).~~

Prenatal exposure to PM has been associated with fetal growth restriction, low birth weight, preterm birth (Gianicolo et al. 2012; Gianicolo et al. 2014), and cause adverse health outcomes in adulthood. Maternal exposure to air pollution has been suggested to adversely affect pregnancy by inducing oxidative stress and inflammation, which may result in impaired placental angiogenesis (van den Hooven et al. 2012). ~~In utero,~~ PM exposure during different periods of gestation affects miRNAs levels. In a recent study of Tsamou and colleagues, miRNA expression was analyzed by qRT-PCR

in 210 placental tissues from mother-newborn pairs. The results indicated that miR-21-5p, miR-146a-5p, and miR-222-3p were inversely associated with PM exposures during the second trimester of pregnancy, while placental expression of miR-20a-5p and miR-21-5p was positively associated with first trimester exposure. In silico prediction tools showed that a~~A~~ common putative target of these miRNAs is the tumor suppressor PTEN, ~~involved in many key cellular processes by negatively regulating PI3K/Akt pathway related to cell survival, cell cycle, angiogenesis, and metabolism (Kitagishi & Matsuda 2013)~~ that was validated measuring its expression by qRT-PCR in a subset of the same cohort. miR-21-5p, miR-20a-5p, and miR-222-3p were inversely correlated with PTEN, confirming the miRNA-PTEN co-expression in placental tissue (Tsamou et al. 2018).

~~Metal rich-PM.~~ PM also contains carcinogenic and toxic heavy metals, which can induce epigenetic modifications. Metals are one of the most important factors causing cardiovascular and respiratory diseases due to systemic activation of pro-inflammatory pathways occurring after exposure (Mercorio et al. 2017).

A cohort study conducted by Bollati et al. analyzed blood miRNA profile of steel plant workers; the samples were collected at the beginning and at the end of the working week. The analysis through qRT-PCR showed that miR-21-5p and miR-222-3p were significantly increased in leukocytes after three high metal-rich PM exposure workdays. Moreover, miR-21-5p level was positively correlated with 8-hydroxy-guanine (8-OH-dG), indicating a relationship with oxidative stress. Also bioinformatic analysis confirmed that these specific miRNAs are involved in pathways related to oxidative stress and inflammation, such as mitogen-activated protein kinase (MAPK) signaling, chemochine signaling pathway, transforming growth factor-β (TGFβ), TLR, and other signaling pathways related to general function (focal adhesion, apoptosis, etc.) (Bollati et al. 2010). Afterwards, another study done again by Bollati et al. investigated whether PM and metal-rich PM alter MVs signaling. The results showed that the expression of miR-128 and miR-302c was significantly overexpressed after three days of workplace PM exposure compared with the beginning of the working week. The authors suggested that these pollutants could affect MVs-associated miRNAs,

representing a novel mechanism of air pollution toxicity. Bioinformatic approaches revealed that nuclear factor kappa B (NFkB) was found to be a central molecule in the networks of both miRNAs, which also regulated gene expression linked with CVD (Bollati et al. 2015). Additionally, the effect of metal-rich PM was evaluated in a subset of the same study population identifying four PM-sensitive miRNAs (miR-29a-3p, miR-146a-5p, miR-421, and let-7g-5p)

~~The effect of metal-rich PM in foundry workers identified four PM-sensitive miRNAs (miR-29a, miR-146a, miR-421, and let-7g)~~ that were differentially expressed in post-exposure compared with baseline samples and seem to be implicated in the inflammatory processes. A quantitative PCR was performed to examine mRNA expression of eighteen predicted target genes of the dysregulated miRNAs. Notably, miR-29a-3p negatively correlates ~~directly interacts~~ with PTEN mRNA, let-7g-5p interacts with NFkB and ~~transforming growth factor-β (~~TGFβ~~)~~, miR-146a-5p targets as many as eight mRNAs, from which a complex network of interactions originates that converge to ultimately influence TGFβ mRNA expression, miR-421 negatively correlates with interferon alpha receptor subunit 2 (IFNAR2) mRNA expression and positively with endothelial nitric oxide synthase (eNOS) and platelet derived growth factor receptor (PDGFR). All of these interactions promote a pro-inflammatory response leading potentially to several diseases, including CVD and respiratory illness (Motta et al. 2013).

The environmental impact on spermatogenesis is also an important issue, influencing male reproductive health. Li et al. assessed expression of miRNAs ~~I~~in spermatozoa of men living in areas polluted from electronic waste compared with men living in a non-polluted site. Microarray analysis identified 182 dysregulated miRNAs and only eleven of these were further validated by qRT-PCR. This analysis showed that miRNA level ~~expression~~ was differentially altered (miR-10b-5p, miR-33b-5p, miR-106a-5p, miR-155-5p, miR-183-5p, miR-205-5p, miR-208a, miR-222-3p, miR-223-3p were up-regulated while miR-363-3p and let-7d-5p were down-regulated) in the polluted group than the control one ~~from men living in a non-polluted site~~. Cluster a~~A~~nalyses ~~showed~~ displayed that miR-10b-5p and let-7d-5p were linked to spermatogenesis, leading to possible male reproductive disorder,

and that the most significant signal was for the Notch signaling pathway (Yan Li et al. 2012) which plays a major role in the regulation of embryonic development and promotes proliferative signaling during neurogenesis (Pierfelice et al. 2011). ~~and that disruption of miR-10b and let-7d effect spermatogenesis, leading to possible male reproductive disorder (Yan Li et al. 2012).~~

Diesel exhaust particles (DEP). Long-term exposures to DEP, the main source of genotoxic substances in urban areas, can contribute to chronic outcomes, such as cardiovascular, metabolic, and respiratory diseases (Rider & Carlsten 2019).

Rider et al. analyzed the bronchial brushings miRNA expression levels of fifteen subjects with atopy carrying out a double-blinded crossover study. The objective of their study was to determine whether exposure to allergen, or DEP, or coexposures modulated miRNA profile that was examined applying Nanostring Technologies' nCounter® assay. They found a weak relationship between miRNAs and DEP exposure, but miR-183-5p, miR-324-5p, miR-132-3p, and miR-331-3p were significantly associated with allergen exposure. Bioinformatic analysis showed a negative correlation both for miR-132-3p with cyclin-dependent kinase inhibitor 1A (CDKN1A) and miR-183-5p with human leukocyte antigen A (HLA-A) (Rider et al. 2016).

~~After DEP exposure, miR-132-3p was up-regulated and miR-183-5p was down-regulated, inducing asthma and other respiratory diseases. miR-183-5p, which was significantly repressed following allergen exposure, targets forkhead box O1 (FOXO1), a transcription factor crucial in responding to oxidative stress. miR-132-3p works as a repressor of cyclin-dependent kinase inhibitor 1A (CDKN1A). It encodes its inhibitor (p21), which regulates cell proliferation by binding to cyclin-CDK complexes. In short, reduced CDKN1A expression could promote dysregulation of cell cycle control, increased epithelial cell apoptosis, and activity of pro-inflammatory pathways (Rider et al. 2016).~~

Adverse health effects associated with DEP exposure could be mediated in part by oxidative stress. Yamamoto and colleagues described an association between miR-144 and oxidative stress. The randomized crossover design of the study included thirteen ~~When~~ subjects with mild asthma ~~were~~

exposed to DEP. miRNA profiling using Nanostring nCounter® assay showed increased levels of miR-21-5p, miR-30e, miR-215, and miR-144 in their peripheral blood. The validation phase by qRT-PCR confirmed a significant up-regulation of miR-144. To investigate the biological function of miR-144, the authors conducted a PCR analysis that showed a negative association of nuclear factor, erythroid-derived 2 (NRF2) and its downstream antioxidant genes with miR-144. The latter was also positively correlated with 8-OH-dG (Yamamoto et al. 2013)., ~~miR-144 was found upregulated in their peripheral blood~~ and thus, ~~associated with oxidative stress. This miRNA targets nuclear factor, erythroid2 like 2 (~~NRF2~~FE2L2),~~ is a transcription factor ~~regulating the cellular responses to oxidative stress (Sangokoya et al. 2010). In fact, NFE2L2~~ that regulates the expression of detoxifying enzymes, determining an adaptive response to oxidant pollutants exposure (Lodovici & Bigagli 2011) ~~(Yamamoto et al. 2013)~~.

Traffic-related air pollution (TRAP). Road transport contributes considerably to air quality problems through vehicle emissions and leads to adverse cardiorespiratory effects including exacerbation of asthma, reduced lung function, myocardial infarction, cardiovascular mortality, and neurodegenerative diseases (Matz et al. 2019).

Krauskopf et al. investigated the relationship between TRAP exposure and miRNAs expression in 24 non-smoking participants using next-generation sequencing technology. In this ~~an~~ randomized experimental crossover study, twenty-four ~~comparison of~~ subjects ~~who~~ walked for 2 hours along Oxford Street in London and then, in a separate session, for other 2 hours through traffic-free Hyde Park. The plasma miRNA profile of the two different sessions was compared~~with that of the same subjects who walked, in a separate session, for 2 hours through traffic-free Hyde Park, miRNA expression pattern showed~~ and decreased levels of miR-27a-5p, miR-133a-3p, miR-145-5p, miR-193b-3p, miR-433-3p, miR-580-3p, miR-6716-3p, and increased levels of miR-1224-5p and miR-3127-5p were observed for the TRAP exposure samples. Further bioinformatic analysis indicated ~~showed~~ that the potential targets of these miRNAs included ~~were~~ genes involved in CVD, respiratory diseases, cancer-related pathways (breast cancer, non-small cell lung cancer, etc.), and signaling

pathways such as the PI3K-Akt and p53 (Krauskopf et al. 2018). ~~As an example, miR-145-5p inhibits growth and migration of breast cancer and it has also been identified to inhibit the proliferation of non-small cell lung cancer cells by targeting the oncogene *c-Myc* , while miR-1224- 5p, silences leucine-rich repeat kinase 2 (LRRK2), a crucial factor known to be down-regulated during pathogenesis of Parkinson's disease (J.-Q. Li et al. 2014)(Krauskopf et al. 2018).~~

Ozone (O3). Ozone is a secondary air pollutant associated with various adverse health effects, predominantly attributable to respiratory diseases (Zhang et al. 2019).

Fry et al. analyzed t~~T~~he sputum of healthy non- asthmatic subjects collected before and after exposure to O_3 for 2 hours ~~was analyzed~~ using a microarray approach. ~~and~~ T~~t~~he results showed that O_3 significantly increased the levels of ten ~~seven~~ miRNAs (miR-25-3p, miR-132-3p, miR-143-3p, miR-145-5p, miR-199a-3p, miR-199b-5p, miR-222-3p, miR-223-3p,~~and~~ miR-434-5p, and miR-582-5p) that, according to computational prediction, are involved in inflammation process and immune-related diseases. ~~In inflammation response, miR-222 is known to be related to neutrophil hyperactivity and granulocyte development targeting myocyte enhancer factor 2C (MEF2C), a transcription factor that promotes myeloid progenitor proliferation, miR-143 plays a role in neutrophil influx , and miR-145-5p is linked to several physiological features of asthma. In immune response, miR-199b-5p directly regulates the nuclear factor of activated T cells (NFAT) pathway , miR-132-3p effects interferon-stimulated gene expression , and miR-434-5p and miR-25-3p regulate immune cell differentiation~~ Moreover, human monocyte-derived macrophages were used in an in vitro model, in order to validate the dysregulation of miR-145-5p and miR-199b-5p and their mRNA targets. Cyclin D1 (CCND1) and v-*myc* avian myelocytomatosis viral oncogene homolog (MYC) showed a significant decreased expression which demonstrate the induced effect of O_3 on these two miRNAs and their targets (Fry et al. 2014).

miRNAs affected by organic chemicals

Polycyclic Aromatic Hydrocarbons (PAHs). Exposure to PAHs, environmental pollutants formed during the incomplete combustion of organic materials, may generates various adverse health outcomes, such as respiratory diseases and some cancers (Kim et al. 2013). Moreover, PAHs could be an additional risk factor for impaired vascular health and atherogenic processes that gradually lead to CVD (Kim et al. 2013; Xu et al. 2013). Results from the human studies concerning miRNA alteration after organic chemical exposure are shown in Table 2.

Insert Table 2 about here.

Ruiz-Vera and colleagues in their cross-sectional study assessed pPlasma levels of vascular-related miRNAs in women exposed to PAHs via biomass combustion smoke (using wood as a fuel source in their house). After qRT-PCR analysis, they found had higher levels of miR-126a-3p and miR-155-5p in this group compared to than women not exposed to PAHs. Bioinformatic analysis to predict the target genes and pathways of miR-126a-3p and miR-155-5p showed a possible relationship with cardiovascular events, notably in the progression of atherosclerosis; in fact the predicted target genes/pathways are linked to inflammation, vascular endothelial health, and other similar pathways (Ruiz-Vera et al. 2019). ~~These miRNAs are important regulators in the progression of atherosclerosis. miR-126 is involved in angiogenesis via vascular endothelial growth factor (VEGF) pathway (Nicoli et al. 2010) and is associated with the vascular smooth muscle cell turnover related to atherosclerotic plaque thinning (Zhou et al. 2013). miR-155 is classified as a pro-inflammatory agent regulating inflammation-related genes and is related to macrophage foam cell formation in atherosclerotic lesions (Nazari-Jahantigh et al. 2015) (Ruiz-Vera et al. 2019).~~

Additionally, PAHs are metabolically activated to form stable PAH-DNA adducts and cause DNA oxidation. This event may lead to DNA damage, a common cause of cancer (Xue & Warshawsky 2005). In this sense, in the study of Deng et al. exposure to relatively high concentrations of PAHs lead to altered miRNAs expressions in previously healthy coke oven workers relative to control groups. ~~Specifically,~~ As a result of PAHs exposure, only miR-150-5p was up-regulated, while ~~the down-regulation of~~ four miRNAs (miR-24-3p, miR-27a-3p, miR-142-5p, and miR-28-5p) were

down-regulated. These four miRNAs regulated genes that could protect against adverse effects of PAH exposure and the increased level of miR-150-5p is linked to a decreased immune response (Deng et al. 2014). ~~miR-24-3p negatively regulates H2AX, which encodes a protein crucial for double- stranded break repair; thus, reduced expression of this gene might increase cellular sensitivity to DNA-damaging agents and genomic instability (Lal et al. 2009). miR-27a-3p can be down-regulated by reactive oxygen species and may enhance transcription factor II human (TFIIH), which is involved in DNA repair processes. In this regard, exposure to PAHs results in an increased DNA repair capacity and decreased chromosome damage . miR-142-5p down-regulation can lead to up-regulation of signaling lymphocytic activation molecule (SLAM)-associated protein (SAP) and to increase T-cell function and IgG production; it may protect individuals against the deleterious effects of PAHs . The down-regulation of miR-28 may elevate the expression of NFE2L2 and may protect cells from DNA damage. As a result of PAH exposure, only miR-150-5p was up-regulated; it is a key regulator of *c-Myb*, an important gene for immune cell differentiation and activation. miR-150-5p deficiency can lead to an enhanced immune response; therefore, miR-150-5p up-regulation may decrease immune response to PAH exposure and make subjects more susceptible to their dangerous effects (Xiao et al. 2007)(Deng et al. 2014).~~

Polychlorinated biphenyls (PCBs). Polychlorinated biphenyls ~~PCBs~~are considered a class of toxic aromatic chemical compounds that bio-accumulate specially in the fatty tissue of humans and animals (Lignell et al. 2016).

Several studies investigated the association between PCBs and miRNAs ~~are linked to increased risk of multiple types of cancer, including non-Hodgkin lymphoma~~ (Engel et al. 2007; Vrijheid et al. 2016).

Krauskopf et al. reported that PCBs exposure can lead to various type of cancer in humans. In their population-based study, PCBs serum levels and other persistent organic pollutants were assessed in healthy subjects and microarray analysis identified a total of 93 miRNAs ~~were found~~ significantly associated (53 positively and 40 negatively) with PCBs exposure. The miRNA profile integration

with transcriptome profile displayed an interaction with oncogenes such as MYC, CCND1, B-cell lymphoma 2 (BCL2) and vascular endothelial growth factor A (VEGFA). The predicted target genes were related to various types of human cancer and involved in signaling pathways like Wnt, apoptosis, and cell cycle regulation (Krauskopf et al. 2017). ~~Among~~The most positively correlated miRNAs, namely ~~these, some miRNAs, such as~~ miR-29a, miR-31-5p, miR-34a-5p, miR-152, and miR-193a-3p, were indicated as tumor suppressors ~~miRNAs~~ (Misso et al. 2014; Liang et al. 2015; Yan et al. 2015; Kim et al. 2015; Liu et al. 2016). ~~for example, miR-29a represses a total of eight gene targets, including the lymphoma-related genes, *CCND1* and *BCL2* (Krauskopf et al. 2017).~~ PCBs exposure may also be attributed to birth defects and embryonic development delays (Vrijheid et al. 2016). Healthy pregnant women living in an area polluted with PCBs who underwent therapeutic abortion due to fetal malformations had PCBs blood concentrations that correlated with miR-191-5p up-regulation compared with women living in a non-polluted area who had a healthy pregnancy. Furthermore, a PCR analysis showed ~~Up-regulation of miR-191 leads to~~ the down-regulation of aryl-hydrocarbon receptor repressor (AHRR), C-terminal binding protein 1 (CTBP1) and Fas cell surface death receptor (FAS) in peripheral blood cells (Guida et al. 2013). miR-191-5p has as sequence complementary to the 3′-UTR region of these genes that are involved in pathways related to immune and inflammatory effects ~~which can~~ induce oxidative stress, immunotoxicity, and cancer (Pradhan et al. 2011; Tao et al. 2012; Yuan-fang Li et al. 2012). Thus, these changes along with the dangerous effects of PCBs ~~in the CTBP1, FAS and AHRR protein expression~~ can interfere with normal development and health conditions. In the study of Li and colleagues, in order to examine the association between miRNAs and some pollutants, healthy placental tissues were collected from a birth cohort. A positive association between PCBs and miR-1537 expression level was found ~~in placental samples~~, suggesting that miRNA profiles may signal in utero exposure to environmental chemicals. ~~The target of miR-1537 is *N-Myc* gene, which regulates cell growth, proliferation, and apoptosis. It encodes MYCN proto-oncogene protein, whose overexpression can lead to tumorigenesis~~(Li et al. 2015).

Phthalates and phenols. Phthalates and phenols are two classes of potential endocrine disrupting chemicals present in the environment, consumer products, and food, which are mainly associated with adverse female fertility outcomes (Vrijheid et al. 2016). In the study of Martinez et al. ~~E~~environmental exposure to phthalates and phenols with EV-miRNA profiles was evaluated in follicular fluid of 130 women who provided urine samples during ovarian stimulation. This cross-sectional study showed an altered expression of e~~E~~ight EV-miRNAs (miR-15b-5p, miR-19a-3p, miR-24-3p, miR-125b-5p, let-7c, miR-106b-5p, miR-374a-5, and miR-375) ~~were found associated~~ linked to ~~with~~ phenol~~s~~ and phthalate concentrations. ~~Specifically, miR-15b-5p, miR-19a-3p, miR-24-3p, miR-125b-5p, and let-7c were up-regulated, while miR-106b-5p, miR-374a-5, and miR-375 were down-regulated.~~ In silico analysis revealed that the potential target genes of m~~M~~ost of these miRNAs might be involved in ~~were associated with~~ follicular development and oocyte maturation and function, highlighting the potential effect of phenol~~s~~ and phthalate~~s~~ exposure on female fertility. Some putative pathways regulated by these miRNAs are TGFβ, phosphoinositide-3-kinase/protein kinase B (PI3K/Akt), forkhead box O (FOXO), MAPK, p53, epidermal growth factor receptor (EGFR), and janus kinase/signal transducers and activators of transcription (JAK/STAT) signaling pathways (Martinez et al. 2019). La Rocca et al. reported that the exposure to phthalates and phenols during pregnancy may influence several biological processes implicated in placental and fetal health. In their study, t~~T~~he associations between first-trimester urine concentrations of phenols and phthalate~~s~~ metabolites and expression of candidate miRNAs in placenta showed a down-regulation of miR-185 in response to phthalate~~s~~ exposure and down-~~-~~regulations of miR-15a-5p and miR-142-3p in response to phenols. These miRNAs, according to in silico analysis, are related with ~~regulate protein serine/threonine kinase~~ Akt activity, ~~a major component of the apoptotic pathway,~~ the insulin like growth factor receptor signaling pathway, metencephalon development, and embryonic epithelial tube formation ~~This indicates that prenatal phthalate and phenol exposure may interfere with several biological processes that have been implicated in placental and fetal healt~~ (LaRocca et al. 2016).

Among phthalates and phenols, bisphenol A (BPA) was reported to affect neurological, cardiovascular, and metabolic diseases (such as diabetes), cancers, and have harmful consequences for the developing fetus (Ikezuki et al. 2002; Jedeon et al. 2013). A cohort of pregnant women exposed to BPA was enrolled in a case-control study carried out by De Felice et al., in order to investigate miRNA changes.~~In placentas from pregnant women exposed to BPA,~~ Microarray analysis showed the altered profile of eighteen miRNAs; the verification of their dysregulated expression by qRT-PCR revealed a significant overexpression of miR-146a-5p ~~was overexpressed compared to a control group~~. Functional significance of this miRNA was evaluated applying bioinformatic analysis and suggested that ~~This~~ miR-146a-5p~~NA~~ is probably associated to neural disease genes (interleukin 1 receptor associated kinase 1- IRAK1, sortilin 1- SORT1 etc.), endocrine system genes pathway (TP53 regulating kinase), cardiovascular disease genes (ABL2) and cancer-related pathways (EGFR, p53, ~~toll-like receptor-~~ TLR) (De Felice et al. 2015).

Organophosphorus pesticides (OP). Organophosphorus pesticides are heavily used in agriculture, but chronic OP exposure is implicated in many adverse health outcomes, such as neurological and respiratory effects (Almeida et al. 2019). In a longitudinal study involving 27 farmworker and non-farmworker adults, Weldon et al. examined the effect of pesticide exposure on u~~U~~rinary miRNAs expression. The samples were collected during two agricultural seasons (thinning and post-harvest).~~s linked to pesticide exposure from 27 parent/child, farmworker/non-farmworker pairs collected during two agricultural seasons (thinning and post-harvest) were assessed. They found s~~ Significant differences in the ~~miRNA~~ profile~~s~~ of six miRNAs (miR-28-5p, miR-133b, miR-223-3p, miR-517b-3p, miR-518d-3p, miR-597) were found in ~~between farmworker and non-farmworker adults, as well as between seasons. Six miRNAs (miR-28-5p, miR-133b, miR-223-3p, miR-517b-3p, miR-518d-3p, miR-597), some of which are involved in neurological functions including neurotransmitter activity and receptor binding, were positively associated with~~adults farmworker~~s~~ ~~status~~ during the post-harvest season, indicating that they may be novel biomarkers of pesticide exposure and early biological response. Bioinformatic analysis identified that miR-28-5p was ~~has been~~ associated with

acetylcholine binding, acetylcholinesterase, and cholinesterase activity and that miR-517b, miR-518d-5p, and miR-597 were associated with target genes involved in neurological functions including neurotransmitter activity and receptor binding (Weldon et al. 2016).

~~miR-223-3p modulates activities of cytochrome P450 family 3 subfamily A member 4 (CYP3A4) and cytochrome P450 family 2 subfamily E member 1 (CYP2E1) genes, which are responsible for the breakdown of many toxic environmental chemicals and carcinogens that enter the body, in addition to basic metabolic reactions such as fatty acid oxidations . miR-133b impairs the expression of glutathione-S- transferase pi 1 (GSTP1), an important factor for fulfilling protective and detoxifying functions in tumor cells miR-517b, miR-518d-5p, and miR-597 are associated with target genes involved in neurological functions including neurotransmitter activity and receptor binding (Weldon et al. 2016).~~

miRNAs affected by heavy metals

Arsenic (As). Human exposure to high As concentration, a toxic metalloid widely distributed in the environment, is related to many health disorders, mainly CVD and cancer (Vrijheid et al. 2016; Rehman et al. 2018). Many epidemiological studies have shown evidence that exposure to inorganic As could have harmful effects on the cardiovascular system of humans, such as ischemic heart failure, cardiac arrhythmias, and endothelial dysfunction (Stea et al. 2014). Results of the described studies are displayed in Table 3.

Insert Table 3 about here.

In a cross-sectional study by Pérez-Vázquez et al. ~~Research has found~~ a significant negative association between urinary As concentration and plasma miR-126-3p levels in Mexican children was found (Pérez-Vázquez et al. 2017). Other studies indicate that miR-126-3p is the most abundant miRNA in endothelial cells that contributes to regulate vascular integrity and developmental angiogenesis besides to be involved in many cardiovascular pathways; this makes miR-126-3p down-

regulation an early biomarker of CVD diseases (Fish et al. 2008; Wei et al. 2013) ~~(Pérez-Vázquez et al. 2017)~~.

Accumulated evidence suggests that the bladder epithelium may be one of the primary targets of As-induced carcinogenesis. Michailidi et al. analyzed ~~U~~urine samples from subjects exposed to different level of As, showing miR-200c-3p and miR-205-5p inversely associated with As exposure compared to unexposed controls. Moreover, the authors validated the expression of these miRNAs in urine samples from patients with urothelial carcinoma in comparison with controls without cancer. The results displayed a low expression of miR-205-5p, suggesting its potential use as biomarker for bladder cancer (Michailidi et al. 2015). Both ~~of these~~ miR-200c-3p and miR-205-5p~~NAs~~ have tumor suppressive functions. miR-200c-3p can reverse epithelial mesenchymal transition via regulation of zinc finger E-box-binding homeobox 1 (ZEB1) and ZEB2 (Wellner et al. 2009). Hence, these miRNAs~~Both miR-200 and miR-205~~ may play a role in the tumor initiation and progression ~~(Michailidi et al. 2015)~~.

A case-control study conducted by Banerjee et al. investigated peripheral blood mononuclear cell (PBMC) miRNA profile of individuals chronically exposed to As through drinking water (experimental group) and in unexposed control. The experimental group was divided into subjects with As induced skin lesions and skin cancer and those without any skin lesions. The ~~L~~levels of miR-21-5p, analyzed by qRT-PCR, were up-regulated in individuals with skin lesions exposed to As compared to the control group and were higher in those with skin lesion than the no-skin lesion subgroup. ~~through drinking water; it~~To our knowledge, this was one of the few studies that experimentally analyzed, by Western blot analysis, miRNA targets (Banerjee et al. 2017). miR-21-5p has been designated as a miRNA associated with carcinogenic outcomes (Sun et al. 2014) due to its direct genes targets, PTEN and programmed cell death 4 (PDCD4)~~,~~ that were found to be inversely correlated to miR-21-5p expression. Again, as expected, the expression of survival protein increased ~~a neoplastic transformation inhibitor (Banerjee et al. 2017)~~. All these results were also validated through an in vitro experiment that showed similar trends (Banerjee et al. 2017).

In another multi-stage study, Sun et al. assessed the levels of 754 miRNAs in the plasma of subjects exposed to As through coal-burning. The expression of 74 miRNAs was dysregulated by using microarray, and twelve were further analyzed by qRT-PCR. The levels of four miRNAs (miR-21-5p, miR-145, miR-155-5p and miR-191-5p) were higher in ~~Differential expression of plasma miRNAs between~~ people exposed to As ~~through coal-burning and~~ compared to a control group. ~~showed an up-regulation of four miRNAs (miR-21, miR-145, miR-155 and miR-191) which~~ Bioinformatic tools showed that these miRNAs inhibit the target genes of pathways linked to oxidative stress, DNA damage repair, and immune inflammation. miR-21-5p targets were MAPK, JAK/STAT, and chemokine pathways which may be involved in chronic arsenic poisoning. The signaling pathways related to miR-145 target genes were MAPK signaling, associated with oxidative stress, and the FOXO signaling pathway, involved in various types of diseases. miR-155-5p and mir-191-5p are involved in changes in the MAPK, PI3K/Akt, and tumor necrosis factors (TNF) pathways (Sun et al. 2017).

In utero exposures to As can harm the developing fetus, increase risk of spontaneous abortions~~disrupt host defenses~~, and lead to deleterious health outcomes (Farzan et al. 2013). Rager et al. conducted a study in which 40 cord blood samples were selected from mother-newbors pairs from a pregnancy cohort exposed to As. ~~Cord blood samples from prenatal exposure to As~~ Microarray analysis revealed~~sulted in~~ an increased expression of twelve miRNAs (miR-16-5p, miR-17-5p, miR-20a-5p, miR-20b-5p, miR-26b-5p, miR-96-5p, miR-98-5p, miR-107, miR-126-3p, miR-195-5p, miR-454-3p, let-7a-5p) associated with As exposure. Then, qRT-PCR was performed, considering only those miRNAs highly involved in disease-associated signaling network, namely miR-107 and miR-26b-5p. The analysis conducted on a subcohort of ten subjects confirmed the microarray results. The twelve analyzed miRNAs, in line with bioinformatic analysis, were linked to~~involved in~~ immune response signaling pathways, such as triggering receptor expressed on myeloid cells 1 (TREM1), TLR, interferon signaling, protein kinase C theta (PRKCQ) signaling in T lymphocytes and B cell receptor signaling (Rager et al. 2014). Some of these miRNAs also have As-related health outcomes including

cancer (let-7a-5p, miR-16-5p, and miR-20b-5p) (Lui et al. 2007; Cascio et al. 2010) and diabetes mellitus (miR-107, miR-126-3p) (Guay et al. 2011). ~~(Rager et al. 2014).~~

Lead (Pb). Lead ~~Pb~~ is a cumulative toxic metal that affects multiple body systems and is associated with numerous toxic events, such as CVD and chronic kidney disease (Rehman et al. 2018).

A cross-sectional study assessed t~~T~~he relation ~~association~~ between heavy metals, microalbuminuria (a marker of vascular and renal damage), and miRNAs ~~was analyzed~~ in adolescents~~, showing that urinary Pb and As levels were correlated with miR-21~~. No relationship between heavy metals and microalbuminuria was found, but urinary Pb and As levels were correlated with miR-21-5p that was also associated with microalbuminuria. ~~It was postulated that this miRNA was protective against the development of albuminuria (a marker of vascular and renal damage) as it inhibited apoptosis of podocytes. But,~~ The results showed that miR-21-5p might be involved in the pathogenetic mechanisms linking heavy metal exposure and albuminuria (Kong et al. 2012).

Exposure to Pb may also contribute to increased risk of spontaneous abortion and preterm delivery, disrupting processes involved in normal development (Silbergeld & Patrick 2005).

Sanders et al. examined the association between metal levels and the expression of 74 miRNAs using Nanostring nCounter®assay. In their cohort study, pregnant women were enrolled. Maternal Pb and mercury (Hg) exposure ~~in the cervix~~ during the second trimester of pregnancy ~~have~~ determined ~~shown~~ a decreased expression of miR-575 and miR-4286 in the cervix cells in relation to Pb levels of tibial bone, while seventeen miRNAs were found to be negatively associated with toenail Hg levels. Functional analysis revealed that ~~T~~th~~e~~se miRNAs associated to Hg were implicated in reproductive system development and morphology, and Pb-associated gene targets were enriched for preeclampsia.~~organismal, cellular, and cardiovascular system development, as well as cell cycle, cancer, and gene expression. Notably, miR-125b, miR-205, and let-7 were involved in various human cancers and directly regulate some oncogenes, including *PTEN*, *p53*, and *RAS*. Other impacted~~ Furthermore, these miRNAs have known impacts on a number of cell cycle and proliferation pathways that could affect parturition and the reproductive system in general. In particular, miR-4286

is predicted to target three genes involved in aryl-hydrocarbon receptor (AHR) signaling pathway (Sanders et al. 2015), which ~~plays a critical role in the~~ has a role in the processes of female reproductive system (Hernández-Ochoa et al. 2009)., ~~including AHR repressor, prostaglandin E synthase 3 (PTGES3), and tumor protein p73 (Sanders et al. 2015). Again,~~ In the study of Li et al., already described above, some miRNAs were altered following Pb exposure in placenta samples. ~~Down-regulation of~~ MiR-10a-5p, miR-146a-5p, miR-190b, miR-431-5p and, let-7f-5p were found to be down-regulated, while miR-651 was up-regulated, suggesting the potential role of miRNAs as markers of prenatal environmental exposures (Li et al. 2015). Literature analysis indicated that miR-146a-5p ~~which~~ targets genes involving in TLR pathway (tumor necrosis factors-receptor associated factors 1- TRAF1 and IRAK1) ~~genes that are involved in TLR pathway,~~ that play a key role in the innate immune response ~~indicates a host-cell-mediated immune activation in response to Pb~~ (Saba et al. 2014). ~~Down-regulation of let-7f plays a critical role in early development, primarily by driving cell differentiation.~~Dysregulation of miR-190b has been speculated to lead to several mental disorders due to its regulation of Neuregulin 3 (NRG3)-mediated inhibitory control processes of the amygdala (Pietrzykowski & Spijker 2014).

Mercury (Hg). Environmental and occupational Hg exposure can occur due to mining and pollution and may cause serious health risks in the cardiovascular, nervous, and immune systems (Vrijheid et al. 2016; Rehman et al. 2018).

The expression of miRNAs in plasma samples was evaluated in a case-control study carried out by Ding and colleagues involving workers occupationally exposed to Hg, divided into chronic Hg poisoning group, Hg absorbing group, and control group in a Hg thermometer plant. The authors used miRNA microarray to investigate the expression of 418 miRNAs in these groups and the results showed that four miRNAs (miR-16-5p, miR-30c-3p, miR-181a-5p and let-7e-5p) were down-regulated and four miRNAs (miR-92a-3p, miR-122-5p, miR-451a and miR-486-5p) were up-regulated in the Hg poisoning group compared to the other two. High levels of miR-92a-3p and miR-486-5p in the mercury poisoned group were confirmed by qRT-PCR (Ding et al. 2016).~~Since Hg~~

~~exposure in female workers affected miR-92a-3p and miR-486-5p expression, these miRNAs could be used as biomarker for Hg exposure.~~ Literature analysis showed that ~~Up-regulation of miR-92a is linked to endothelial dysfunction which causes the formation of atherosclerotic lesions targeting eukaryotic ribosome biogenesis protein (ERB1); up-regulation of~~ miR-486 may be~~is~~ linked to inflammatory diseases through the enhancement of NFkB activation associated with Hg poisoning (Song et al. 2013). ~~(Ding et al. 2016).~~

Several studies have found that high prenatal exposure to Hg for reproductive females has been related with increased preterm birth risk and other adverse birth outcomes (Silbergeld & Patrick 2005). In the aforementioned study of Li et al., ~~S~~seventeen miRNAs were down-regulated in response to high levels of Hg in human placentas, including some let-7 family members which may indicate a state of disrupted placental development in response to chemical exposures (Li et al. 2015).

Chromium (Cr). Chromium~~r~~ is naturally found in rocks and soil, it can be liquid, solid or gas and exists in various oxidation states. Epidemiological studies have suggested that Cr exposure may be linked with metabolic diseases and CVD (Rehman et al. 2018).

Dioni et al. analyzed the leukocytes miRNA expression levels of ninety obese subjects. In the screening phase 43 miRNAs were negatively associated with Cr levels, and only ten miRNAs were chosen for the validation phase. Among these, ~~N~~nine miRNAs (miR-451a, miR-301, miR-15b, miR-21-5p, miR-26a-5p, miR-362-3p, miR-182, miR-183-5p and miR-486-3p) confirmed the same trend of the first phase. Functional analysis identified the top canonical pathways for these miRNAs, namely molecular mechanisms of cancer, axonal guidance signaling, protein kinase A (PKA) signaling, role of nuclear factor of activated T cells (NFAT) in cardiac hypertrophy PTEN signaling. ~~were down-regulated in association with Cr exposure including miR-451 and miR-486-3p~~ In particular, miR-486-3p was positively associated with blood pressure and may be a factor risk in CVD due to its interaction with PTEN pathway, as also identified by bioinformatic analysis; on the contrary, miR-451a ~~which~~ was~~ere~~ negatively linked to glycated hemoglobin (HbA1c) (Dioni et al. 2017); therefore it ~~and blood pressure, respectively. miR-451~~ could have a role in the diabetes

pathogenesis because the increases in HbA1c indicate a decreased control of blood glucose levels (Soliman et al. 2014).~~; it also regulates p38 MAP kinase signaling~~. ~~miR-486-3p may be a factor in CVD risk due to its interaction with PTEN pathway (Dioni et al. 2017).~~

~~A~~ The study conducted by Li et al., on 117 workers in a chromate production plant in China, showed a significant inverse association of high Cr exposure with plasma miR-3940-5p level, which was also linked to micronuclei frequency (Li et al. 2014), a biomarker of DNA damage (Bonassi et al. 2005). In addition to bioinformatic analyses, an enzyme-linked immunosorbent assay was performed, in order to quantify the protein expression levels of miR-3049-5p-mediated genes. The results showed that miRNA-3940-5p regulates the X-ray repair cross complementing 2 (XRCC2) gene (Li et al. 2014) which is ~~has been~~ implicated in DNA repair mechanisms (Serra et al. 2013). ~~and may increase this activity when reaching a certain exposure of Cr.~~Hence, t~~T~~his result demonstrates that miR-3940-5p can play a modulatory role in Cr-induced genetic damage ~~(Li et al. 2014)~~.

Other metals. The association between several metals and PAHs with miRNAs expression was analyzed in a case-control study involving 360 healthy male coke oven workers. The expression of many plasma miRNAs was found to be negatively associated with aluminum, antimony, Pb, and titanium, and positively associated with molybdenum and tin. This study demonstrated a relationship between some miRNAs and biomarkers for genetic damage and oxidative stress, such as micronuclei and 8-OH-dG ~~8- hydroxydeoxyguanosine~~(Deng et al. 2019). Among the analyzed miRNAs, some have important functions. ~~In particular, l~~Let-7b-5p can regulate the expression ~~levels~~ of genes deputated to DNA-repair ~~genes~~ (Spolverini et al. 2017) and it is involved in p53-regulated pro-apoptotic pathway and nucleotide excision repair pathway (Saleh et al. 2011; Encarnación et al. 2016). miR-126-3p plays a role ~~is implicated~~ in cancer-related processes, such as inflammatory responses (Zampetaki & Mayr 2012). ~~and cellular protection against reactive oxygen species imbalance.~~ Improper expression of miR-16-5p can negatively affect DNA repair mechanism influencing ~~by modulating~~ the expression of DNA damage-related proteins (Patel et al. 2017). Finally, miR-320b is known to be down-regulated in human cancers; its target is TP53 regulated inhibitor of

apoptosis 1 (TRIAP1) through which may control apoptosis process ~~and may exert its activity on apoptosis targeting TP53 regulated inhibitor of apoptosis 1 (TRIAP1)~~ (Li et al. 2016). These findings may suggest a potential linkage ~~mechanistic connection~~ between the ~~complex~~ metal-PAH complex interactions and the early harmful effects on human health (Deng et al. 2019).

Discussion ~~Conclusions~~ and future directions

This review reports and aggregates the literature evidence on the effects of environmental chemical exposure ~~to~~ causinge miRNA dysregulation in humans and consequently the alteration of several biological pathways. This may provide~~s~~ possible explanations for ~~links between exposure and~~ disease pathogenesis.

We observed a great heterogeneity in body samples, methodologies for analysis of miRNAs and their targets, and study designs, so that it is difficult to directly compare such different studies.

With regard to the sample type adopted for the miRNA analysis, we found that blood (and its components like serum, plasma, and PBMC) was the most used, maybe because of its numerous advantages. miRNAs are highly stable into the bloodstream where are released in a quantity that allows their collection for testing. They derive from target tissues (i.e., brain, kidney, lung) and may reflect their status; in fact, the miRNA expression pattern in tissue and blood is similar (Powrózek et al. 2020). In the blood, the different expression of miRNA in pathological condition may be used as a disease biomarker, since it helps to discriminate between patients and healthy subjects. This allows obtaining information on the health condition and the organ physiology, suggesting the high utility of blood, and especially of plasma, for diagnostic, predictive and prognostic purposes (Panagopoulos & Lambrou 2018; Iacob et al. 2020). However, among the different body fluids, the expression pattern of miRNAs may change. Moreover, the extraction methodology (for example the extraction of miRNAs incorporated in MVs may yeld different results) and the platform employed can enhance these differences, making difficult miRNAs comparison and functional analysis.

The different analytical methods used for identifying miRNAs may cause differences in their detection levels, indicating the need for a standardized approach to miRNA analytical processing. qRT-PCR was the most adopted instrument of analysis, as it is considered the "gold standard" because of its higher precision and sensitivity, rapidity and ease of use (Hunt et al. 2015); it is often used to validate results from the microarray platform. The latter is cheaper than qRT-PCR but has a low dynamic range in detection and a low specificity (Heller 2002). Another rarely adopted methodology is Nanostring Technologies' nCounter® platform, a hybridization-based method that provides a simple solution for multiplexed detection of up to 800 miRNAs in a single reaction, but it is expensive (Mathew et al. 2020), as is next-generation sequencing that is capable of concurrently detecting new miRNAs (Hunt et al. 2015). As observed in the analyzed studies, since qRT-PCR can detect only annotated miRNAs, the sequencing approach should be more considered because it could allow the discovery of novel miRNAs to be used as biomarkers.

For a biological interpretation of miRNA function, most studies performed bioinformatics analysis, and in some cases also a literature research. This analysis allowed selection of miRNAs with a putative biological function, paving the way for further experimental and clinical investigations that might confirm the consistency and, possibly, the reasons of the observed associations. In fact, only a few investigations (Guida et al. 2013; Motta et al. 2013; Yamamoto et al. 2013; Fry et al. 2014; Y. Li et al. 2014; Banerjee et al. 2017; Tsamou et al. 2018) included in vivo or in vitro experiments in order to better understand the molecular mechanisms triggered by miRNAs after environmental exposures. In such limited cases, it was possible to identify those genes and pathways regulated by specific miRNAs, which, in turn, may determine a pathological condition.

With respect to the different study designs, we have found that some studies have a cross-sectional nature that does not allow identification of the causal association between environmental exposure and the alteration of the miRNA profile.

We explored the coherence of miRNA expression (up-regulation or down-regulation) in response to a certain pollutant or within the same category of pollutants (air pollution, organic chemicals, heavy metals). As for PM, the altered level of some miRNAs (miR-21-5p, miR-222-3p, let-7 family members) was observed in several studies but with a differential direction of expression (Bollati et al. 2010; Yan Li et al. 2012; Motta et al. 2013; Yamamoto et al. 2013; Louwies et al. 2016; Rodosthenous et al. 2016; Tsamou et al. 2018). On the contrary, if we look at the whole category (air pollution), two miRNAs, miR-223-3p and miR-132, were found up-regulated in response to most airborne pollutants (PM, O_3, DEP) (Yan Li et al. 2012; Fry et al. 2014; Rodosthenous et al. 2016). In relation to the organic chemicals, the dysregulated expression of common miRNAs within the same pollutant was not found. On the contrary, referring to the whole category, we noticed an altered expression of miR-24 and miR-28 in response to most organic chemicals (PAHs, OP, phthalates and phenols), but with a different direction of expression (Deng et al. 2014; Weldon et al. 2016; Martinez et al. 2019). Concerning the heavy metals, miR-21-5p was observed to be up-regulated after As exposure in some studies (Banerjee et al. 2017; Sun et al. 2017), while miR-126 was common in two studies but with different regulations (Rager et al. 2014; Pérez-Vázquez et al. 2017). Considering the whole category, miR-21-5p was found up-regulated not only in response to As but also after exposure to Pb (Kong et al. 2012).

~~We have observed that miR-21-5p and let-7 family are those most involved in the response of environmental contaminations (PM, Cr, As, DEP, Pb, Hg, phthalates and phenols) and modulate oxidative stress, immune inflammation, tumor suppression, DNA damage repair, and apoptosis. Cardiovascular pathology, metabolic diseases, respiratory diseases, and child development are the most critical areas, due to the effects on the inflammation pathway; when inflammation becomes chronic, inflammatory factors can lead to cancer. We have also observed that studies investigating the same environmental factor identified distinct miRNAs. For metal rich-PM and As, miR-21-5p was up-regulated in some studies and down- regulated in others (Table 1).~~

~~**Table 1**~~

In addition to the reasons listed above, t~~T~~he~~is~~ conflicting results ~~heterogeneity~~ may be partly explained by ~~the different tissues and cell types used in the analysis,~~ the different exposure durations between various studies, and the effects of age, gender, and health~~y~~ status of the participants.

Insert Table 4 about here.

When compiling a summary to identify the most relevant miRNAs, our review showed that the most reported miRNAs, whose expression is associated to environmental contaminants, are miR-21-5p, miR-222-3p, miR-223-3p, and let-7 family members. Table 4 shows the biological effects and the health implications of these miRNAs. The predicted target genes are involved in pathways related to inflammation, oxidative stress, cell cycle regulation, apoptosis, and tumorigenesis. In particular, PTEN, MAPK, and NFkB are the pathways most involved. As for health implications, cardiovascular and respiratory diseases, and child development are the most critical areas, due to the effects on the inflammation pathway; when inflammation becomes chronic, inflammatory factors can lead to cancer.

Hence, i~~I~~t ~~will~~ could be useful ~~necessary~~ to deeply investigate the biological processes involving miR-21-5p, miR-222-3p, miR-223-3p, and let-7 family ~~biological processes~~ that may ~~which~~ lead to the development ~~onset~~ of several diseases, and this could help further analysis to establish ~~in order to identify new~~ potential therapeutic approaches in NCDs management. Nevertheless, these miRNAs showed to be sensitive to multiple pollutants and this condition somewhat reduces their specificity as exposure biomarker for a given toxic substance. For this reason, it would be needed to search for miRNAs that more specifically respond to a certain contaminant.

Conclusions

Our review identified numerous miRNAs dysregulated after environmental exposure, although there are few studies conducted in humans. In some cases, important advances have been made in relation to the associations between specific miRNAs and biological responses to environmental risk factors.

However, large-scale supplementary investigations are mandatory to identify the actual causative roles. In addition, in vitro or in vivo experiments could be integrated into the study design, in order to have more consistent results. Indeed, an integrated analysis, combining miRNA expression and mRNA expression of critical related genes, may be important to understanding the mechanisms under genetic damage.

Further efforts should be made to systematize the scientific evidence, both analyzing miRNAs that specifically intervene in a given disease in relation to a particular pollutant and identifying miRNAs that ~~which~~ may be used as possible exposure biomarkers.

Diclosure of interest

The authors report no conflict of interest.

Financial diclosure

The authors have no funding to disclose.

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Table 1. Human studies on miRNAs dysregulation after air pollution exposure and their targets

miRNA	Methodology	miRNA expression	Sample type*	Study design	miRNA target analysis	Signaling pathways or target genes	Contaminant	Reference
21-5p, 223-3p	qRT-PCR	↓	Blood	Cohort	Bioinformatics analysis	HMGB, PTEN	PM	Louwies et al. 2016
15a-5p, 19b-3p, 23a-3p, 93-5p, 126-3p, 130-3p, 142-3p, 146a-5p, 150-5p, 191-5p, 223-3p, let-7a-5p, let-7g-5p	Nanostring nCounter®assay	↑	Serum	Cohort	Bioinformatics analysis	Genes linked to CVD-related pathways	PM	Rodosthenous et al. 2016
20a-5p, 21-5p (1st trimester)	qRT-PCR	↑	Placenta	Cohort	Bioinformatics analysis	PTEN	PM	Tsamou et al. 2018
21-5p, 146a-5p, 222-3p (2nd trimester)		↓			qRT-PCR	PTEN (miR-21, miR-20a, and miR-222)		
21-5p, 222-3p	qRT-PCR	↑	Blood leukocytes	Cohort	Bioinformatics analysis	MAPK, TGFβ, FAK, TLR	Metal rich-PM	Bollati et al. 2010
128, 302c	qRT-PCR	↑	Plasma	Cohort	Bioinformatics analysis	NFKB	Metal rich-PM	Bollati et al. 2015
182 miRNAs	Microarray	Dysregulated	Blood	Cohort			Metal rich-PM	Motta et al. 2013
29a 146a 421	qRT-PCR	↑			qRT-PCR	PTEN TGFβ IFNAR2, eNOS, PDGFR		
let-7g						NFKB, TGFβ		
182 miRNAs	Microarray	Dysregulated	Spermatozoa	Case-control			Metal rich-PM	Li et al. 2012
10b-5p	qRT-PCR	↑			Bioinformatics analysis	BCL2L11, DAZAP1, BCL6, Notch		
33b-5p, 106a-5p, 155-5p, 183-5p, 205-5p, 208a, 222-3p, 223-3p						Notch		
let-7d-5p 363-3p		↓				YBX2 Notch		
132-3p	Nanostring nCounter®assay	↑	Bronchial epithelial cells	Randomized double-blinded crossover	Bioinformatics analysis	CDKN1A	DEP	Rider et al. 2016
183-5p		↓				HLA-A		

21-5p, 30e, 215, 144 144	Nanostring nCounter®assay	↑ ↑	Peripheral Blood	Randomized double-blinded crossover	PCR	NRF2	DEP	Yamamoto et al. 2013
1224-5p, 3127-5p 27a-5p, 133a-3p, 145-5p, 193b-3p, 433-3p, 580-3p, 6716-3p	Next-generation sequencing	↑ ↓	Plasma	Randomized crossover	Bioinformatics analysis	PI3K-Akt, p53	TRAP	Krauskopf et al. 2018
25-3p, 132-3p, 199a-3p, 222-3p, 434-5p, 582-5p 143-3p, 223-3p 145-5p, 199b-5p	Microarray	↑	Induced sputum	Experimental	Bioinformatics analysis N/P in vitro model	Inflammatory pathways CCND1, MYC	O_3	Fry et al. 2014

*Sample type is referred to miRNAs detection
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;
N/P, not performed

Table 2. Human studies on miRNAs dysregulation after organic chemicals exposure and their targets

miRNA	Methodology	miRNA expression	Sample type*	Study design	miRNA target analysis	Signaling pathways or target genes	Contaminant	Reference
126a-3p, 155-5p	qRT-PCR	↑	Plasma	Cross-sectional	Bioinformatics analysis	Genes liked to cancer, inflammation, apoptosis, vascular endothelial health	PAHs	Ruiz-Vera et al. 2019
150-5p	qRT-PCR	↑	Plasma	Cross-sectional	Bioinformatics analysis	Genes linked to immune response	PAHs	Deng et al. 2014
142-5p 24-3p 27a-3p 28-5p		↓				Genes linked to DNA damage		
93 miRNAs	Microarray	53 ↑ 40 ↓	Serum	Cohort	Bioinformatics analysis	MYC, CCND1, BCL2, VEGFA	PCBs	Krauskopf et al. 2017
191-5p	qRT-PCR	↑	Peripheral blood	Case-control	PCR	AHRR, CTBP1, FAS	PCBs	Guida et al. 2013
1537	Nanostring nCounter®assay	↑	Placenta	Cohort	N/P		PCBs	Li et al. 2015
15b-5p, 19a-3p, 24-3p, 125b-5p, let-7c	Microarray	↑	Follicular fluid	Cross-sectional	Bioinformatics analysis	TGFβ, PI3K/Akt, FOXO, MAPK, p53, EGFR, JAK/STAT	Phthalates and phenols	Martinez et al. 2019
106b-5p, 374a-5p, 375		↓						
185 (phthalates) 15a-5p, 142-3p (phenols)	qRT-PCR	↓	Placenta	Cohort	Bioinformatics analysis	Akt, insulin like growth factor receptor signaling, embryonic epithelial tube formation	Phthalates and phenols	La Rocca et al. 2016
18 miRNAs	Microarray	Dysregulated	Placenta	Case-control			Phthalates and phenols	De Felice et al. 2015
146a-5p	qRT-PCR	↑			Bioinformatics analysis	IRAK1, SORT1, TP53, ABL2, EGFR, p53, TLR		
28-5p,	qRT-PCR	↑	Urine	Nested case-control	Bioinformatics analysis	acetylcholinesterase and cholinesterase activity	OP	Weldon et al. 2016
517b-3p, 518d-3p, 597						genes involved in neurological functions		
133b, 223-3p,						N/S		

*Sample type is referred to miRNAs detection;
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;

N/P, not performed;
N/S, not specified

Table 3. Human studies on miRNAs dysregulation after heavy metals exposure and their target

miRNA	Methodology	miRNA expression	Sample type*	Study design	miRNA target analysis	Signaling pathways or target genes	Contaminant	Reference
126-3p	q-RT-PCR	↓	Plasma	Cross-sectional	Literature analysis	N/S	As	Pérez-Vázquez et al. 2017
200c-3p, 205-5p	q-RT-PCR	↓	Urine	Case-control	Literature analysis	N/S	As	Michailidi et al. 2015
21-5p	q-RT-PCR	↑	PBMC	Case-control	Western blotting	PTEN, PDC4	As	Banerjee et al. 2017
74 miRNAs	Microarray	56 ↑ 18 ↓	Plasma	Case-control			As	Sun et al. 2017
21-5p 145 155-5p 191-5p	qRT-PCR	↑			Bioinformatics analysis	MAPK, JAK/STAT, chemokine pathway MAPK, FOXO MAPK MAPK, PI3K-Akt, TNF		
16-5p, 17-5p, 20a-5p, 20b-5p, 26b-5p, 96-5p, 98-5p, 107, 126-3p, 195-5p, 454-3p, let-7a-5p	Microarray	↑	Cord blood	Cohort	Bioinformatics analysis	TREM1, TLR, PRKCQ	As	Rager et al. 2014
26b-5p, 107	qRT-PCR	↑						
21-5p	qRT-PCR	↑	Urine	Cross-sectional,	Literature analysis	N/S	Pb	Kong et al. 2012
575, 4286	Nanostring nCounter® assay	↓	Cervix cells	Cohort	Bioinformatics analysis	Pathways linked to cell cycle and proliferation, like AHR	Pb	Sanders et al. 2015
146a-5p 190b 10a-5p, 431-5p, let-7f-5p 651	Nanostring nCounter® assay	↓ ↑	Placenta	Cohort	Literature analysis	N/S	Pb	Li et al. 2015
17 miRNAs		↓				N/S	Hg	
16-5p 30c-3p 181a-5p let-7e-5p	Microarray	↓	Plasma	Case-control	Literature analysis	N/S	Hg	Ding et al. 2016
92a-3p 122-5p 451a		↑						

486-5p 92a-3p 486-5p	qRT-PCR	↑						
15b, 21-5p, 26a-5p, 362-3p, 182, 183-5p, 451a, 486-3p	qRT-PCR	↓	Blood-leukocytes	Cohort	Bioinformatics analysis	PTEN, axonal guidance and PKA signaling	Cr	Dioni et al. 2017
18 miRNAs	Microarray	13 ↓ 3 ↑	Plasma	Case-control			Cr	Li et al. 2014
590-5p 3940-5p	qRT-PCR	↓			N/P Enzyme- linked immunosorbent assay	XRCC2		
71 miRNAs	Solexa sequencing	Dysregulated	Plasma	Case-control			(Other metals):	Deng et al. 2019
16-5p, 24-3p, 27a-3p, 28-5p, 126-3p, 142-5p, 150-5p, 320b, 451a, let-7b-5p	qRT-PCR	↓			Literature analysis	N/S	Antimony	
16-5p, 320b		↓					Aluminum	
27a-3p		↓					Pb	
126-3p		↑					Molybdenum	
16-5p, 24-3p, 27a-3p, 28-5p, 126-3p, 142-5p, 320b, let-7b-5p		↑					Tin	
24-3p, 27a-3p, 28-5p, 126-3p, 320b, let-7b-5p		↓					Titanium	

*Sample type is referred to miRNAs detection;
↓ / ↑, difference in miRNAs expression, respectively up- and down-expressed;
N/S, not specified;
N/P, not performed

Table 4. List of promising miRNAs involved in environmental exposure, biological function and health implication

miRNA	Environmental exposure	Signaling pathway or target genes	Biological function	Health implication	Reference
21-5p	PM, Metal rich-PM, As, Cr	PTEN (inhibitor of PI3K-Akt)	Tumor suppressor, inflammation	CVD, birth defects, cancer	Bollati et al. 2010; Louwies et al. 2016; Banerjee et al. 2017; Dioni et al, 2017; -Sun et al. 2017; Tsamou et al. 2018
		MAPK	Inflammation, oxidative stress, cell proliferation, differentiation, cell survival and apoptosis		
		JAK/STAT	Inflammation, oxidative stress, cell survival and proliferation		
		p53	Cell proliferation and apoptosis		
		TGFβ	Embryonal development, cellular differentiation, inflammation, immune response		
222-3p	PM, Metal rich-PM, O_3	PTEN (inhibitor of PI3K-Akt)	Tumor suppressor, inflammation	CVD, birth defects, reproductive disorders, respiratory diseases, cancer	Bollati et al. 2010; Li et al. 2012; Fry et al. 2014; Tsamou et al. 2018
		MAPK	Inflammation, oxidative stress, cell proliferation, differentiation, cell survival and apoptosis		
		NFkB	Inflammation, immune response, cell proliferation, tumorigenesis		
223-3p	PM, Metal rich-PM, O_3	PTEN (inhibitor of PI3K-Akt)	Tumor suppressor, inflammation	CVD, male reproductive disorders, respiratory diseases, cancer	Li et al. 2012; Louwies et al. 2016; Rodosthenous et al. 2016
		NFkB,	Inflammation, immune response, cell proliferation, tumorigenesis		
Let-7 family	PM, Metal rich-PM, Phthalates and phenols, Hg, Other metals	PTEN (inhibitor of PI3K-Akt)	Tumor suppressor, inflammation	CVD, male reproductive disorders, adverse female fertility outcomes, respiratory diseases, cancer	Motta et al. 2013; Li et al. 2012; Ding et al. 2016; Rodosthenous et al. 2016; Deng et al. 2019; Martinez et al. 2019
		MAPK	Inflammation, oxidative stress, cell proliferation, differentiation, cell survival and apoptosis		
		JAK/STAT	Inflammation, oxidative stress, cell survival and proliferation		
		NFkB	Inflammation, immune response, cell proliferation, tumorigenesis		
		p53	Cell proliferation and apoptosis		

TGFβ	Embryonal development, cellular differentiation, inflammation, immune function