

# Benzene exposure is associated with epigenetic changes (Review)

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**Abstract.** Benzene is a volatile aromatic hydrocarbon solvent and is known as one of the predominant air pollutants in the environment. Chronic exposure to benzene is known to cause aplastic anemia and increased risk of acute myelogenous leukemia in humans. Although the mechanisms by which benzene causes toxicity remain to be fully elucidated, it is widely accepted that its metabolism is crucial to its toxicity, with involvement of one or more reactive metabolites. Novel approaches aimed at evaluating different mechanisms by which benzene can impact on human health by altering gene regulation have been developed. Among these novel approaches, epigenetics appears to be promising. The present review article summarizes the most important findings, reported from the literature, on epigenetic modifications correlated to benzene exposure. A computerized search in PubMed was performed in November 2014, using search terms, including 'benzene', 'epigenetic', 'histone modifications', 'DNA methylation' and 'microRNA'. Epidemiological and experimental studies have demonstrated the potential epigenetic effects of benzene exposure. Several of the epigenomic changes observed in response to environmental exposures may be mechanistically associated with susceptibility to diseases. However, further elucidation of the mechanisms by which benzene alters gene expression may improve prediction of the toxic potential of novel compounds introduced into the environment, and allow for more targeted and appropriate disease prevention strategies.

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## 1. Introduction

Benzene is a volatile aromatic hydrocarbon solvent and an important industrial chemical, which is present in petroleum products and combustion effluents. Owing to its chemical properties, benzene is known as one of the most common air pollutants in the environment (1). In the past, benzene was used predominantly as a solvent, however, occupational exposure in industrialized countries occurs through the production and use of petroleum derivatives, in particular fuels, in various settings, including factories, refineries, rubber production plants, shoe manufacturing and printing factories (2-4). The general population is exposed to benzene through inhalation; exposure sources include motor vehicle emissions, gasoline evaporative loss at service stations and cigarette smoke (5,6). In addition, the consumption of contaminated foods or water can present a secondary route of exposure (7). The exposure to benzene can produce various health effects; available reports have suggested that occupational exposure to high doses of benzene can induce hematotoxicity, aplastic anemia and leukemia (8-11). Several studies have examined the possibility that benzene causes leukemia at high concentrations; however, it has since been suggested that exposure to levels <10 ppm increases the risk of acute and chronic leukemia (11-14). Although the mechanisms by which benzene causes toxicity remain to be fully elucidated, it is generally accepted that its metabolism is critical in the toxicity, with involvement of one or more reactive metabolites (15,16). The metabolism of benzene predominantly occurs in the liver, where the P4502E1 cytochrome catalyzes the introduction of a single oxygen atom forming benzene oxide. However, previous studies have suggested that it is also metabolized and covalently bound *in situ* in bone marrow (17-20). Although benzene metabolism remains to be fully elucidated, numerous *in vitro* and *in vivo* studies have indicated that the primary products are phenol, catechol and hydroquinone, the majority of which are excreted in urine as glucuronide and sulphate conjugates (17,21). However, catechol and hydroquinone can also be partially transformed

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**Abbreviations:** AML, acute myelogenous leukemia; DNMT, DNA methyltransferase; VOCs, volatile organic compounds

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into reactive o- and p-benzoquinone, respectively. Benzene is also converted to a ring-opened oxidation product, t,t-muconic acid, via the precursor, t,t-muconaldehyde. In addition, benzene oxide can be a substrate of glutathione-S-transferase, forming mercapturic acid. There is evidence that hydroquinone, phenol, catechol, benzoquinone and muconaldehyde are actually involved in benzene toxicity; whereas the glutathione conjugate of mercapturic acid, and the glucuronide and sulphate conjugates of hydroquinone, catechol, phenol and benzoquinone are the results of detoxifying processes (22-24). These benzene metabolites are different in chemical structure and reactivity, therefore, it is difficult to determine which may contribute the most to producing benzene toxicity or leukemia. t, t-muconaldehyde and 1,4-benzoquinone are the most potent in inhibiting erythropoiesis, followed by hydroquinone and catechol, co-administration of phenol with hydroquinone and catechol, and certain glutathione adducts of hydroquinone (17). Furthermore, benzene exposure can cause damage to the immunological, neurological, and reproductive systems (25-27).

Previous studies have assessed the different mechanisms by which benzene can alter gene expression and cause toxicity in humans. Among these, epigenetics appears to be a promising tool. Epigenetics is the investigation of mitotically and meiotically heritable changes in gene expression without alterations in the DNA sequence. Several environmental factors have been associated to aberrant changes in epigenetic pathways in experimental and epidemiological studies (28-30). As these epigenetic changes are small, potentially cumulative and may develop over time, it may be difficult to identify the cause-effect links among environmental factors, epigenetic changes and diseases, and the role of environmental exposure in epigenetic alterations remains to be fully elucidated (28,31,32).

Certain epigenetic mechanisms, including DNA methylation, histone modifications and microRNA (miRNA) expression, can alter genome function under the effects of several factors, including chemical and environmental toxins, age, gender or lifestyle. Epigenetic modifications may mediate specific mechanisms of toxicity and responses to certain xenobiotics. In addition, these alterations may persist even following removal of the triggering factor (33,34).

The present review article summarizes the most important findings, reported from the literature, on epigenetic modifications correlated to benzene exposure (Table I).

## 2. Methods

In the present review, a computerized search in PubMed was performed in November 2014, using search terms, including 'benzene', 'epigenetic', 'histone modifications', 'DNA methylation' and 'microRNA'. Studies published in English, Italian and Chinese were included. The studies were selected according to the following criteria: Original study or meta-analyses; studies providing information on the association between benzene exposure and epigenetic modifications. Studies, which included detailed occupational exposure assessment, rather than those based solely on job titles were prioritized for selection. There were no restrictions with regard to the place of origin or the ethnicity of the subjects. The only restriction was to studies published in English, which reported the risk

associated with benzene *per se*; risk estimates for benzene grouped with similar chemicals, for example other aromatic hydrocarbons, were excluded.

## 3. Epigenetic mechanisms

In the present review, three classes of epigenetic modifications were described: DNA methylation, post-translational histone modifications and miRNA expression.

DNA methylation is the most widely investigated epigenetic marker, which consists of the catalytic addition of a methyl group to the fifth carbon position of a cytosine residue, is followed on the same strand by guanine, and is also known as a CpG dinucleotide. Although their presence in the genome is rare, CpG dinucleotides occur in concentrations known as CpG-islands, which can be found in the promoter region of ~50% of all human genes (29). In normal cells, the promoter CpG-island regions are characteristically non-methylated (29,35). CpG hypermethylation at promoter regions causes loss of function, whereas global hypomethylation at repetitive sequences causes genomic instability. These two types of alteration can contribute to a disease phenotype (28).

The second class of epigenetic changes includes the post-translational modification of the 15e30 amino acid N-terminal unstructured histone tails. These modifications, involving the methylation of lysine and arginine residues, acetylation of lysine residues, phosphorylation of serine and threonine residues, ADP-ribosylation and ubiquitylation, can alter chromatin condensation, and all these modifications alter DNA transcription (28,36). The functional consequences of these modifications depend on the specific amino acid involved and on the specific covalently bound group; acetylation determines the loosening of chromatin and leads to replication and transcription, whereas methylated histones tighten DNA and restrict access to various enzymes (37).

miRNAs are short single-stranded RNAs of ~20-24 nucleotides in length, which are transcribed from DNA but not translated into proteins. miRNAs negatively modulate the expression of target genes at the post transcriptional level by binding to the 3' untranslated regions of target mRNAs. Each mature miRNA is partially complementary to multiple target mRNAs, and directs the RNA-induced silencing complex to identify the target mRNAs for inactivation (38). Since the miRNA activity was confirmed in experimental and epidemiological studies involving animals and humans, miRNAs have gained significant attention due to their important modulatory effect on biological functions, including cellular proliferation, apoptosis, differentiation, metabolism and development. miRNAs also can chromatin structure and are involved in the maintenance of genome stability (39,40).

The direct interaction between miRNAs and epigenetic modifications is considered to be a complex system. The expression of miRNAs is tissue-specific, and is accurately regulated by epigenetic mechanisms, including DNA methylation and histone modifications. By contrast, miRNAs can also alter epigenetic mechanisms, control gene transcription and target post-transcriptional gene-silencing (41).

**DNA methylation.** Aberrant DNA methylation models include global hypomethylation, gene-specific hypermethylation and

Table I. Epigenetic modifications induced by benzene and its metabolites.

Substance	Epigenetic changes	Type	Reference
Benzene	LINE-1 and Alu DNA hypomethylation; gene-specific hypermethylation of CDKN2B; hypomethylation of MAGE1	Human	32
	Altered methylation induced by benzene at several CpG sites; hypomethylation of RUNX3 (AML2) gene; hypermethylation of MSH3 gene and <i>Sema3C</i> protein	Human	34
	Downregulation of the expression of p15 and p16 modulated by DNA promoter methylation	Human	35
	LINE-1 and Alu DNA hypomethylation; Hypomethylation of STAT3	Human 37	33
Hydroquinone and 1,4-benzoquinone	Hypermethylation of IL12 gene; hypomethylation of RUNX1T1 and MAGE-1 genes;	<i>In vitro</i>	34
	Global DNA hypomethylation	36	
Benzene	Histone modifications	Mouse	38
Benzene	Upregulation of miR-154*, miR-487a, miR-493-3p and miR-668;	Human	34
	Detected expressions of miR-638, let-7f-5p and miR-223-3p;	Human	39
	Upregulation of miR-34a, miR-205, miR-10b, let-7d, miR-185 and miR-423-5p-2; downregulation of miR-133a, miR-543, hsa-miR-130a, miR-27b, miR-223, miR-142-5p and miR-320b;	Human	40
	69 miRNAs significantly differentially expressed in volatile organic compound-exposed samples. vs. controls;	Mouse	41
	Association between maternal expression of miR-223 and indoor concentrations of benzene; high expression levels of miR-223 associated with lower regulatory T cell numbers in maternal and cord blood	Human	42

miR, microRNA; LINE1, long interspersed element 1; CDKNB1 cyclin-dependent kinase inhibitor 2B; MAGE1, melanoma-associated antigen 1; RUNX2, runt-related transcription factor 2; MSH3, MutS homolog 3; STAT3, signal transducer and activator of transcription 3; RUNX1T1, runt-related transcription factor 1 translocated to 1.

hypomethylation; these changes are frequently reported in AML and other cancer tissues (41). It has been demonstrated significant long interspersed element 1 leukocyte and Alu DNA hypomethylation, as well as gene-specific hypermethylation of cyclin-dependent kinase inhibitor 2B and hypomethylation of melanoma-associated antigen 1, is found in workers exposed to low doses of benzene (42). These results indicate that low-level benzene exposure may produce altered DNA methylation, resembling the aberrant epigenetic patterns of malignant cells. It was also found that DNA methylation, measured in specimen repeats collected at intervals of 8 years,

decreased more markedly in exposed subjects, compared with control subjects, and an association of DNA epigenetic modifications in subjects exposed to low doses of benzene was demonstrated (43). A preliminary study using DNA extracted from the peripheral blood mononuclear cells of six workers exposed to benzene and four control individuals showed gender-specific methylation patterns for numerous genes, and also found altered methylation induced by benzene at several CpG sites (44).

A previous study involving 11 patients with benzene poisoning demonstrated that the average methylation level of

p16, but not of p15, was higher than that in the control group. This result suggested that benzene may negatively alter the expression of p15 and p16 through DNA methylation. Patients with benzene poisoning exhibiting p15 and p16 promoter DNA methylation are more likely to develop AML, myelodysplastic syndrome and lymphoma (45).

To improve comprehension of the carcinogenic capacity of benzene, previous studies have investigated whether exposure to benzene and its metabolites modify the global DNA methylation status in human normal hepatic L02 cells. The alterations induced by the variation of DNA methyl transferase (DNMT) activity were also examined in an HaeIII DNMT-mediated methylation assay *in vitro*. The results indicated that hydroquinone and 1,4-benzoquinone produced global DNA hypomethylation with a statistically significant difference, compared with the control ( $P < 0.05$ ). These data suggested that hydroquinone and 1,4-benzoquinone can disturb global DNA methylation, and that global DNA hypomethylation induced by 1,4-benzoquinone may act through the inhibiting effects of DNMT activity at 10 IM ( $P < 0.05$ ) (46).

By combining DNA methylation and mRNA expression data, Yang *et al* characterized three hypermethylated genes showing concurrent downregulation and two hypomethylated genes showing increased expression. It was hypothesized that aberrant hypomethylated signal transducer and activator of transcription 3 may arise from chronic benzene poisoning (47).

**Histone modifications.** Modified proteins have been detected in the liver and bone marrow of mice following treatment with 155 and 800  $\mu\text{g/kg}$  b.w. [ $^{14}\text{C}$ ] benzene. The findings confirmed the high reactivity of benzene metabolites and their extensive binding to proteins. Detailed investigation of the histones demonstrated that their attack by reactive benzene species is broad, resulting in multiple modified sites within a single histone, and that the degree of  $^{14}\text{C}$  incorporation is independent of the specific histone (48).

**miRNAs.** A preliminary study analyzed the miRNA expression levels in seven exposure-control matched pairs, and identified the upregulation of four miRNAs (miR-154\*, miR-487a, miR-493-3p and miR-668) in the benzene-exposed subjects. Upregulation in the expression of miR-154\*, possibly due to methylation and acetylation in the 14q32 region, has been reported in patients with acute promyelocytic leukemia bearing the t (22;17) translocation (44).

In a previous study, differentially expressed miRNAs in the plasma of benzene-exposed workers were examined, and the potential roles of plasma miRNAs in the development of hematologic toxicity induced by benzene exposure were investigated. Three significant classes of differentially expressed miRNAs were found using cluster analysis. The expression levels of miR-638, let-7f-5p and miR-223-3p, determined using reverse transcription-quantitative polymerase chain reaction analysis, was consistent with the microarray data. Pathway analysis showed that the most enriched pathway was focal adhesion, with six potential functional targets, including son of sevenless homolog 2, vinculin, cyclin D2, collagen type IV  $\alpha 6$ , insulin-like growth factor 1 and mitogen-activated protein kinase 1 (49).

In chronic benzene poisoning, six upregulated miRNAs (miR-34a, miR-205, miR-10b, let-7d, miR-185 and miR-423-5p-2) and seven downregulated miRNAs (miR-133a, miR-543, hsa-miR-130a, miR-27b, miR-223, miR-142-5p and miR-320b) were observed, compared with healthy controls ( $P \leq 0.05$ ). The aberrant miRNA expression levels may be a potential biomarker of chronic benzene poisoning (50).

A previous study investigated whether miRNA expression profiles in the lungs of mice are modified by volatile organic compounds (VOCs) on 44 male Kunming mice. The mice were placed four similar static chambers, comprising a control group and three groups exposed to different doses of a VOC mixture. The results of the study indicated that inhalation of VOCs altered the miRNA patterns that regulate gene expression, potentially leading to the initiation of cancer and inflammatory diseases (51). Herberth *et al* analyzed the expression levels of miR-155 and miR-223, together with regulatory T (Treg) cell numbers in maternal blood during pregnancy, and in cord blood. An association was found between the maternal expression of miR-223 and the indoor concentrations of benzene and toluene, and also demonstrated that maternal tobacco smoke exposure during pregnancy was correlated with the expression level of miRNA-223 in the blood, with an effect on Treg cell numbers in the cord blood and subsequent allergy risk (52).

#### 4. Conclusions

In the present review, the potential effects of benzene on the epigenome were evaluated. Occupational or environmental exposure to this solvent can produce epigenomic changes, which are associated with an increased susceptibility to the development of diseases (36). Whereas several studies have examined DNA methylation, few have considered the effects of environmental pollutants on histone modifications and miRNAs. Further investigations are required in order to assess the mechanisms by which environmental chemicals can modulate the epigenetic cellular setting. It is important to improve current knowledge on the effects of benzene exposure and the epigenetic alterations associated with several diseases. Further elucidation of the mechanisms by which benzene alters gene expression is likely to improve comprehension of the toxic potential of novel environmental pollutants, and to identify more appropriate preventative measures.

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