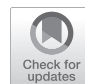

9 Epigenetic Mechanisms in Understanding Nanomaterial-Induced Toxicity

Manosij Ghosh, Lode Godderis, and Peter Hoet

Abstract

The toxic effects of different forms of nanomaterials comprise a series of biological effects such as oxidative stress; DNA damage; inflammatory response; activation of nuclear transcription factors. Some of these are key characteristics of human carcinogens and have been considered for hazard identification of nanomaterials. In addition, epigenetic changes also play a key role in the multi-step sequential process of carcinogenesis. Epigenetic modifications may constitute changes in DNA methylation, histone modifications (methylation, acetylation etc), and changes in non-coding RNA, leading to an altered gene expression profile. In this chapter, we describe the state-of-the-art of epigenetic modifications induced by different nanomaterials, from a limited number of in vitro- in vivo and human studies, a majority of which is primarily focused on DNA methylation. We also highlight the potential challenges and future directions in the field of epigenetics research in nanomaterial toxicology.

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Keywords

Nanotoxicology · Epigenetics · DNA methylation · Histone modification

9.1 Introduction

Different aspects of nanotoxicology has been discussed in the previous chapters, and it has become abundantly clear that nanoparticles (NPs) can induce oxidative stress [30, 53, 60, 75], DNA damage [30, 59, 76], alter DNA repair efficiency [11, 91], induce an inflammatory response [75, 76], can potentially be immunomodulatory/immunosuppressive [13, 42, 62, 66] and can affect cell death/cell proliferation [1, 21, 48, 72, 79, 95]. While, it is also clear that the field of nanotoxicology research is quickly emerging and there are significant knowledge gaps, some of the observed effects reported for one or more nanoparticle align with the "*Key Characteristics of Carcinogens as a Basis for Organizing Data on Mechanisms of Carcinogenesis*" described by Smith et al. [92]. In addition, the "*10 key characteristics*" identified by Smith et al. [92] towards organization of mechanistic data, also included epigenetic alterations.

Epigenetic alterations are defined (by NCI) as changes "in the chemical structure of DNA that does not change the DNA coding sequence" [19]. In other words, epigenetic alterations are broadly considered heritable changes that do not

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H. Louro, M. J. Silva (eds.), *Nanotoxicology in Safety Assessment of Nanomaterials*, Advances in Experimental Medicine and Biology 1357, https://doi.org/10.1007/978-3-030-88071-2_9

involve a change in the DNA sequence itself. Epigenetic alterations, such as DNA/RNA methylation, histone modifications (methylation, acetylation), non-coding RNA regulate gene expression by regulating chromatin structure and accessibility. Such alterations/signatures play a crucial role in proper cellular function, differentiation, development and are influenced by a wide range of endogenous and exogenous factors. One of the most studied epigenetic alteration is DNA methylation (and demethylation), where a methyl-group (CH_3) is covalently added to position 5 of the cytosine pyrimidine ring, and a family of DNA methyltransferase (DNMT) and ten-eleven translocation (TET) enzymes play important role in the maintenance of methylation pattern [40, 46, 100]. It is generally accepted that CpG islands (regions with a high frequency of CpG sites) in promoter regions of genes are predominantly unmethylated which activate gene transcription [4, 5]. Besides, DNA methylation, histone modifications play crucial role in maintaining chromatin conformation, and these processes are tightly coupled [12, 27, 40]. These post-translational covalent modifications of histone mainly studied in the tail domains of H3 and H4, include modifications such as acetylation and methylation of lysine and arginine among other [12, 27, 40]; and are regulated by enzyme families such as histone acetyl transferase (HAT), histone deacetylase (HDAC), Histone methyltransferase (HMT). Another group of key regulators are the non-coding RNAs and regulate gene expression at the transcriptional and post-transcriptional levels [26, 99], and closely interact with the DNA methylation and histone modification machinery [41, 102].

From the standpoint of environmental and occupational diseases and that of chemical induced carcinogenesis, it is considerably well established that epigenetic modifications play a crucial role. Epigenetic changes induced by genotoxic environmental carcinogens have been systematically reviewed by Chappell et al. [14]. While epigenetic changes can be used as biomarker of exposure, or as marker of disease and disease progression [52] they can also be used as potential therapeutic targets [50]. This is particularly relevant for NP toxicology, where rate of NP production far exceeds the hazard identification and risk assessment, while an increasing number of workers and consumers are being exposed to these diverse group of materials [8, 38].

Despite considerable progress in the field of epigenetics and disease biology and cancer epigenetics, major gaps remain in understanding of chemical induced epigenetic alterations. This is also true for the field of nanotoxicology. This chapter will therefore summarize the state-of-the-art and developments in understanding NP induced epigenetic changes and comment briefly on future direction. While the NPs discussed in the chapter is not exhaustive, it is representative of some of the most produced and studied particles in relation to toxicology. We have identified studies that have evaluated one or more epigenetic endpoints, however no mechanistic conclusion for individual particle or NP as a group has been made in the chapter due to the limited number of comparable studies (differences in particle properties, experimental design, test system and cell types).

9.2 Epigenetic Changes Induced by Metal and Metal Oxide Nanoparticles

While the evidence stream on epigenetic changes induced by metal and metal oxide nanoparticle are relatively scarce, some studies have observed epigenetic modifications induced by TiO2-NPs, ZnO-NPs, CuO-NPs, SiO2 NPs, silver and gold nanoparticles among others. In this section we discuss briefly, the distinct mechanistic evidence exhibited by each of these NPs and at the same time try to identify the commonality exhibited by these particles.

9.2.1 TiO2 Nanoparticles

TiO2-NPs are one of the most abundantly produced nanomaterials, with considerable evidence regarding oxidative stress, cyto-genotoxicity, impaired DNA repair efficiency and limited evi-

dence on immunotoxicity, in vitro and in vivo [20, 39, 74, 88, 10, 63]. While limited by number, in this section (Table 9.1), we discuss the growing number of studies that have identified some of the underlying epigenetic mechanism.

Pogribna et al. [80], studied global (5-mC) and gene specific (EpiTect array) changes in DNA methylation, induced by TiO2-NP (Aeroxide TiO2 P25; 100 μg/mL) in Caco-2, HepG2, NL20 and A-431 cell lines. The authors observed global hypomethylation after 72 h of exposure, for A-431, HepG2 and Caco-2 cells and promoter specific methylation changes in several genes associated with apoptosis and cell cycle modulatory effects. Changes were observed in all cell line for *CDKN1A* and *SCARA3*, while genes like *GADD45A*, *DNAJC15*, *TP53* were also differentially methylated in one or more of the cell lines [80]. In the same study, while the expression of the epigenetic regulators (*DNMT1*, *DNMT3A*, *DNMT3B*, *MBD2*, *UHRF1*) were altered, it was not consistent among the cell types. Given that there is epigenetic heterogeneity among tissues and cell types, such studies using multiple cell line (of relevance to the exposure route, and target) can be very important.

Despite evidences of the influence of TiO2 crystal phase on toxicity, the effect of crystal phase on epigenetic changes is not well studied. In our study we reported significant global DNA hypomethylation (5-mC), and a decrease in global hydroxymethylation (5-hmC) induced by different crystal phase of TiO2-NP (NM-102/Anatase, NM-104/Rutile, and NM-105/Anatase-rutile) at sub cytotoxic concentrations in 16-HBE cells [32] however such changes were not mechanistically linked to other toxicity endpoints. In another study the authors used surfaced coated TiO2-NP, to study the effect on DNA methylation, in A549 cells, in addition to other cyto-genotoxic endpoints [93]. The authors observed significant decrease in Long Interspersed Nuclear Element-1 (LINE-1) methylation after 72 h of exposure to silica and citrate coated TiO2-NP, while no significant changes were observed after 48 h of exposure.

Patil et al. [77] also reported global DNA (5-mC) hypomethylation, in MRC-5 cells after 24 and 48 h exposure to TiO2-NP (<100 nm, mixture of rutile and anatase, 1 and 8 μg/mL). They also observed reduced mRNA expression of DNA methyltransferases *DNMT1*, *DNMT3A*, and *DNMT3B* [77]. These changes in DNA methylation were associated with induction of oxidative stress. The influence of TiO2-NP (<25 nm, 24 h) induced oxidative stress on DNA methylation was also reported by Bai et al. [2], in A549 cells, where they observed significant DNA hypermethylation in the promoter region of *PARP*-1 (encoding poly (ADP-ribose) polymerase 1, involved in DNA repair). Based on the other endpoints (cyto-genotoxic and oxidative stress), Stoccoro et al. [93] suggested that oxidative stress could be a primary event in inducing genotoxicity and epigenotoxicity. While most studies have evaluated DNA methylation as a primary epigenetic endpoint, Jayaram and Payne [45], studied the effect of TiO2-NP induced intracellular superoxide on *HDAC9* (histone deacetylase 9) expression, they used SOD-TiO2-NPs (SOD corona), and passivated TiO2-NP. Both passivated and SOD particles did not result in intracellular ROS production. SOD-TiO2-NPs were able to scavenge superoxide and the expression of HDAC9 were comparable to control. Similar results were observed for the passivated TiO2-NPs [45]. In a study by the same group, a decrease in *HDAC9* expression, and an increase in *HDAC10* expression was observed; induced by food-grade TiO2 particles [44]. While food grade TiO2 particle, more specifically E171 may not be primarily a nanoparticle, electron microscopy analysis shows that at least 36% of the particles (by number) are below 100 nm [29, 97]. In another study [57], in addition to oxidative stress, the authors observed significant global DNA hypomethylation in A549 and 16HBE cells at different concentrations (0.1–100 μg mL − 1, 48 h) of TiO2-N25 and TiO2-A60. Both TiO2-N25 and TiO2-A60 exposure resulted in significant changes in the mRNA and protein expression levels of methylation related genes (*DNMT3B*, *TET1*, *TET2* and *TET3*) in A549 and 16HBE

Table 9.1 Summary table of studies reporting epigenetic changes induced by TiO2 nanoparticles

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
JRC TiO2; NM-102 (~21 nm, Anatase; BET surface area 60.227 m^2/g), NM-104; (~26 nm, rutile, BET surface area 41.216 m^2/g), NM-105 (~261 m, Anatase-rutile, BET surface area 37.345 m^2/g);	In vitro	Human Bronchial Epithelial cell line (16HBE)	3.125, 12.5 and 25 μg/ml; 1, 3 and 24 h	Global DNA methylation and hydroxymethylation (LC–MS/MS)	Significant global DNA (5-mC) hypomethylation, decrease in hydroxymethylation	[31]
TiO2-NP (NM101; 20.99 ± 6.4 nm)	In vitro	Human bronchial epithelial cells (BEAS-2B cells)	11 and 20 mg/mL for up to 4 weeks; exposure media changed every 3 days, sample collected at 2 and 4 weeks	Genome wide methylation- human methylation450 BeadChip	DNA methylation changes of 21 CpG sites- corresponding to 22 genes	[90]
Aeroxide P25 (21 nm)	In vitro	Human colorectal adenocarcinoma cells (Caco-2), human hepatocyte carcinoma (HepG2), human bronchial transformed epithelial cells (NL20), human epidermoid carcinoma cells (A-431)	100 μg/mL (non-cytotoxic) TiO2 for 24 or 72 h	Global DNA methylation (5-mC), gene specific methylation-EpiTect methylation array, expression of *DNMT1*, *DNMT3a*, *DNMT3b*, *MBD2*, *UHRF1*	Global DNA (5-mC) hypomethylation, promoter specific hypermethylation of *CDKN1A* and *SCARA3*	[80]
TiO2 (634662)- Sigma Aldrich; <100 nm; mixture of rutile and anatase	In vitro	Human fetal fibroblasts (MRC5)	1 and 8 μg/mL, 24 and 48 h	Immunochemical staining and ELISA based detection of DNA methylation (5-mC); DNMT expression (*DNMT1*, *DNMT3a*, *DNMT3b*)	Global DNA (5-mC) hypomethylation, reduced DNMT activity, oxidative stress	[77]
22.1 nm- Sigma-Aldrich; surface area of 45–55 m^2/g	In vitro	Human lung adenocarcinoma cell line (A549)	6.25, 12.5, 25, 50, 100 μg/mL; 24 h	Methylation-specific PCR	Oxidative stress associated hypermethylation of PARP-1 promoter region	[2]
Pristine TiO2 (5 nm), citrate coated TiO2 and silica coated TiO2, TiO2 Aeroxide® P25 (21 nm)	In vitro	Human lung adenocarcinoma cell line (A549)	8.3–266.6 μg/ml (1.25 to 80 $\mu g/cm^2$); 40 μg/cm2 used for DNA methylation study	ELISA method to detect LINE-1 methylation	Global methylation (of LINE-1) not affected at 48 h, significant hypomethylation at 72 h	[93]

TiO2-NPs (21 nm, #718467, Sigma-Aldrich); P-TiO2-NPs (passivated TiO2-NPs- sodium aluminate, #11138491, Sigma-Aldrich; and silica #1056212500, Sigma-Aldrich); SOD-TiO2-NPs (TiO2-NP with SOD corona)	In vitro	Human lung adenocarcinoma cell line (A549)	800 μg/mL in T25 flask, 994 μg/mL in 35 mm optical dishes; 24 h	*HDAC9* expression (western blot)	Decrease in *HDAC9* expression; the SOD corona inhibits the production of intracellular ROS- partially restoring HDAC9 expression, similar results were observed for passivated TiO2-NP	[45]
Food-grade TiO2 particles (E171), 148 ± 44 nm; up to 32% particles <100 nm (information from literature)	In vitro	Human lung adenocarcinoma cell line (A549)	994 μg/mL	*HDAC9*, *HDAC10* expression (western blot)	*HDAC9*, *HDAC10*	[44]
TiO2-N25 nanotubes (25 nm average diameter, Sigma-Aldrich); TiO2 (TiO2-A60; Anatase, 60 nm average diameter, Aladdin-Shanghai, China)	In vitro	Human lung adenocarcinoma cell line (A549), human bronchial epithelial cell line (16HBE)	Stock further diluted in cell culture media to 0.1, 1, 10 and 100 μg mL − 1 and sonicated within 5 min before the treatment; 48 h	Global DNA methylation (5-mC)	Global DNA hypomethylation, significant changes in the mRNA and protein expression (*DNMT3b*, *TET1*, *TET2* and *TET3*)	[57]
TiO2 (EVONIK-Parsippany, NJ), 21 nm; specific surface area 50 m^2/g	In vitro	Human monocyte (THP1), primary small airway epithelial cells (SAEC), murine macrophage cells (RAW264.7)	The cells were in the respective cell culture media at two doses (0.5 and 30 μg/mL) for 24 h	Global DNA methylation (5-mC) and hydroxymethylation (5-hmC), LINE1 and Alu/SINE methylation specific PCR	No significant difference in global of sequence specific DNA methylation	[55]

(continued)

Table 9.1 (continued)

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
TiO2-NPs (25 nm; anatase, Aladdin-Shanghai, China)	In vivo	Healthy male NIH mice	5-week (young group) 10-week (adult group); housed in controlled conditions (20 ± 2 °C; 50–70% humidity; 12 h light/dark cycle), fed commercial pellet diet and deionized water ad libitum. 4 groups (6 mice/ group): Young control, young TiO2-NP, adult control, adult TiO2-NP; intranasal instillation (20 mg/kg body weight), left naris, once/ day for 30 days	Global DNA methylation (5-mC) and DNA hydroxymethylation (5-hmC) (ELISA); Pyrosequencing (*IFN*-γ, *TNF*-α and *Thy*-1) in lung tissue; *DNMT3B* or *MBD2* expression (western blot); RNA sequencing (Illumina HiSeq 4000)	Increased inflammation; global hypomethylation, decreased DNA hydroxymethylation; significant changes in promoter methylation of *TNF*-α and *Thy*-1; altered mRNA expression-pathways in cancer	[58]
TiO2-NP	Human population	DNA from leucocyte	Non exposed (n = 43, age: 37.95 ± 8.94) TiO2-NP exposed (n = 26, age: 34.65 ± 7.99, exposure duration: 2.33 ± 2.49 years)	Global DNA methylation (5-mC; HPLC)	No significant change in DNA methylation, increased oxidative stress	[54]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

cells. While, most studies have observed oxidative stress and changes in DNA methylation induced by TiO2-NP, in one study [56] the authors did not observe significant changes in global DNA methylation/hydroxymethylation or LINE1 methylation in TiO2-NP (21 nm) exposed THP-1, SAEC, RAW264.7 cells at subtoxic concentrations (0.5 and 30 μg/mL for 24 h). Studies reporting the effect of long-term exposure to TiO2-NP on epigenetic changes are limited. Sierra et al. [90] observed changes in DNA methylation (21 CpG sites- corresponding to 22 genes), in TiO2-NP (NM-101; 20.99 ± 6.4 nm) exposed BEAS-2B cells after a period of up to 4 weeks. Since the number of differentially methylated genes were few, further enrichment analysis was not performed on the set.

In an in vivo study [58], the effect of anatase TiO2-NP (25 nm), after 30 day-intranasal instillations in young (5-week) and adult (10-week) mice was investigated and observed that the lung of young mice were more susceptible, compared to the adult mice. While, inflammatory markers were upregulated in both young and adult mice, significant global DNA hypomethylation and decrease in DNA hydroxymethylation were observed in young mice. Significant changes in sequence specific methylation of *TNF*-α promoter (2 positions) and *Thy*-1 (cell surface antigen) were also observed. Expression of DNMT mRNAs (*DNMT1*, *DNMT3A* and *DNMT3B*) were significantly upregulated in the young mice as well. Changes in DNA methylation, DNMT expression, and changes in expression of genes associated with pathways in cancer indicated the importance of epigenetic regulation and the potential to be used as biomarker for exposure and disease [58]. Additionally, in a cross-sectional study, Liou et al. [54], investigated the effect of TiO2 exposure in workers handling (n = 26) TiO2-NP and observed an increase in oxidative stress, but no changes in global DNA methylation were observed.

Overall, the studies reporting epigenetic alterations induced by TiO2-NP, primarily investigated DNA methylation changes. Despite the heterogeneity in particle and cell types used is such studies, evidence indicate towards disruption of DNA methyltransferase (DNMT) activity and DNA hypomethylation, often associated with increased oxidative stress. Since oxidative stress and oxidative DNA damage has been shown to interfere with the ability of DNMTs to efficiently interact with DNA [104], resulting in DNA hypomethylation and genomic instability, oxidative stress induced epigenetic mechanisms warrant further investigation.

9.2.2 Zinc and ZnO Nanoparticles

Previous studies have identified ZnO-NP induced cyto-genotoxicity and oxidative stress, in vitro and in vivo, and a major attribute of ZnO-NP toxicity is ionization/dissolution [96, 101]. However, only a handful of studies have investigated epigenetic alteration. In a study (Table 9.2) which also evaluated epigenetic effect of TiO2-NP, Patil et al. [77] reported DNA hypomethylation and changes in mRNA expression of *DNMT1*, *DNMT3A*, and *DNMT3B*, in MRC-5 cells for ZnO-NP (<100 nm, 1 and 8 μg/mL, 24 and 48 h exposure). Choudhury et al. [84] investigated the effect of ZnO-NP (90 ± 2 nm) in human embryonic kidney cells (HEK-293) on several key endpoints and observed a significant increase in oxidative stress and apoptosis. The authors observed a significant DNA hypomethylation, sequence specific demethylation of LINE1 and an increase in DNA hydroxymethylation, and a significant increase in expression of Ten-Eleven Translocation (TET) [84]. Another in vitro study in HaCaT cells, exposed to ZnO-NP revealed significant increase in H3K9 methylation and a decrease in H4K5 acetylation [28]. These changes were associated with an increase in expression of pro-apoptotic genes and increased oxidative stress and DNA damage and G2/M cell cycle arrest. While, the limited number of studies provide some evidence regarding oxidative stress induced epigenetic alteration, further studies (both in vitro and in vivo) are required to evaluate the epigenetic changes induced by ZnO-NP taking into account physicochemical attributes such as dissolution.

Table 9.2 Summary table of studies reporting epigenetic changes induced by Zinc nanoparticles

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	Referrences
	Type	System	Condition			
ZnO-NP (544906)- Sigma Aldrich, <100 nm	In vitro	Human fetal fibroblasts (MRC5)	1 and 8 μg/mL, 24 and 48 h	Immunochemical staining and ELISA based detection of DNA methylation (5-mC); DNMT expression (*DNMT1*, *DNMT3a*, *DNMT3b*)	Global DNA (5-mC) hypomethylation, reduced DNMT activity, oxidative stress	[31, 32]
ZnO-NP (90 ± 2 nm), synthesized from 0.5 M zinc nitrate hexahydrate [Zn (NO3)·6H2O] and NaOH	In vitro	Human embryonic kidney cells (HEK-293)	25 and 50 μg/ml, 48 h	Global DNA methylation (5-mC) and DNA hydroxymethylation (5-hmC); expression of TET (*TET1, TET2*, and *TET3*) and DNMT (*DNMT1*, *DNMT3A*, and *DNMT3B*); sequence specific DNA methylation (pyrosequencing)- LINE-1, *NBL2* and *D4Z4*	Significant global DNA (5-mC) hypomethylation, significant increase in hydroxymethylation (5-hmC), reduced DNMT activity, significant increase in TET expression, hypomethylation of LINE1, oxidative stress	[84]
ZnO-NP(<100 nm; 99.7% metal basis; specific surface area, 15–25 m2/g, sigma-Aldrich co. St Louis, MO, USA)	In vitro	Human epidermal keratinocyte cell line (HaCaT)	20, 50 μg/mL; 24 h	Histone modification (H4K5ac, H3K9me2; western blot)	Decreased H4K5ac levels and increased H3K9me2	[28]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

9.2.3 Silica Nanoparticles

Only few studies have reported epigenetic alterations induced by silica nanoparticles (Table 9.3). One such evidence comes from the study by Seidel et al. [86] in Bhas 42 cell, exposed to Min-U-Sil® 5 (crystalline silica, 15 and 25 μg/cm^2) or NM-203 (pyrogenic amorphous silica, 2 and 5 μg/cm^2). The authors observed significant global DNA hypomethylation and significant changes in the DNMT expression (increased *DNMT3A* and *DNMT3B*) [86] and an increase in Histone H4 acetylation and significant changes in HDAC expression by Min-U-Sil® 5, but not for NM-203. For Min-U-Sil® 5, the authors also observed increased acetylated histone H3 lysine 4 (H3K4Ac), histone H3 lysine 9 (H3K9Ac) and acetylated histone H3 lysine 27 (H3K27Ac) on c-Myc promoter, which plays important role in cellular metabolism and proliferation and in cell transformation [86]. Epigenetic alterations were also studied in HaCaT cells, exposed to Sio2 NP (15 nm) [36]; where the authors observed significant increase in promoter methylation of Poly [ADP-ribose] polymerase 1 (*PARP-1*), which plays key role in chromatin remodelling and multiple DNA damage repair pathways [82]. Using a *DNMT1* knockdown, the authors confirmed the role of *DNMT1* in the observed Sio2 NP mediated effect. Sio2 NP exposure (5 μg/mL, 30 passage) in BEAS-2B cells also induced significant changes in DNA methylation including *PI3K-Akt* and sequence specific hypermethylation of *CREB3L1* and *Bcl-2* promoters indicating the role of mitochondria mediated apoptosis [105]. In a cross-sectional study Liou et al. [54], observed an increase in oxidative stress markers in a group of workers handling SiO2 (n = 31), but changes in DNA methylation was not observed. Based on these studies, it can be suggested that Sio2 NP can induce epigenetic changes to the DNA and histone, and these changes are primarily associated with cell cycle regulation and proliferation. However, important aspect of SiO2 NP toxicity such as the crystal structure and surface silanols have not been addressed by these studies.

9.2.4 Copper and Copper Oxide Nanoparticles

In an in vitro study [56], the authors evaluated effect of CuO NP (58.7 nm, 0.5 and 30 μg/m, 24 h) in different cell lines (THP-1, SAEC, RAW264.7) and observed small but significant changes in LINE 1 methylation, while no changes in global DNA methylation/hydroxymethylation were observed (Table 9.4). In a study aimed at identifying anticancer effect of green synthesized CuO NP, the authors observed significant inhibition of HDAC family [47] in A549 cells, however description of exposure was not very well defined for further interpretation. Most evidence regarding epigenetic changes induced by copper and copper oxide nanoparticles come from in vivo studies. While several key endpoints were evaluated, here we discuss the epigenetic endpoints of interest. In an in vivo study [55] on BALB/c male mice, using the same CuO NP (58.7 nm) particle as mentioned above [56], the authors observed an Increase in 5-mC levels and 5-hmC levels in lung tissue, hypermethylation of SINE B1 elements in the alveolar macrophages; and methylation of LINE-1 remained unchanged. In their study on female ICR (Institute of Cancer Research) mice, Rosner et al. [83] observed no significant change in global DNA methylation upon whole-body inhalation exposure to CuO NPs (3 days, 6 weeks, and 3 months). Enriched pathways, based on results of miRNA -mRNA expression changes were associated with lysosomal function, adherens and tight junction, pathways in cancer among others. In another study, Ognik et al. [67] observed an increase in oxidative stress markers (lipid peroxides, MDA), a decrease in catalase, total glutathione and global DNA hypomethylation for animals receiving Cu, CuNP through their diet. Through their results the authors suggested that Cu deficiency could impair oxidative defence, and that CuNP supplementation in the diet reduces protein oxidation, nitration, DNA oxidation and methylation [67].

Table 9.3 Summary table of studies reporting epigenetic changes induced by Silica nanoparticles

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
SiNP	In vitro	Human bronchial epithelial cells (BEAS-2B cells)	5 μg/mL, 30 passages	Genome wide methylation-human methylation450 BeadChip; pyrosequencing	223 CpG hypomethylated, 1973 CpG hypermethylated; PI3K/Akt signaling pathway altered, sequence specific hypermethylation of CREB3L1 and Bcl-2 gene	[105]
SiO2-NP (15 nm; Wan Jing New Material Co. Ltd. Hangzhou, Zhejiang, China)	In vitro	Human epidermal keratinocyte cell line (HaCaT)	HaCaT cell with or without DNMT1 knock down; cells exposed to 2.5, 5, 10 μg/mL SiO2 NP, 48 h	Methylation specific PCR and bisulfite sequencing of *PARP1* promoter	Increase in the level of PARP-1 methylation, decrease of PARP1 expression on mRNA and protein level; expression and promoter methylation of PARP1 restored following DNMT1 knock down	[36]
Min-U-Sil® 5 (crystalline silica particle), NM-203 (pyrogenic amorphous silica nanoparticle); particles dispersed in sterile water, sonicated for 5 min at 10% amplitude (Branson Sonifier S-450 D, Branson Ultrasonics crop., Danbury, CT);	In vitro	Bhas 42 cell	Min-U-Sil® 5 (15 and 25 μg/cm2), NM-203 (2 and 5 μg/cm2); cell in experiment for upto 29 days, cell pellets collected on day 6, and cell pellets were collected on day 29.	Global DNA methylation (5-mC); DNMT (*DNMT1*, *DNMT3A*, *DNMT3B*) and HDAC (*HDAC1*, *HDAC2*, *HDAC3*, *HDAC6*) expression, H3/ H4 acetylation, c-Myc expression and histone modification on c-Myc promoter	Global DNA hypomethylation (Min-U-Sil® 5), increased *DNMT3A* and *DNMT3B* protein levels for Min-U-Sil® 5, no changes observed for NM-203; increase in histone H4 acetylation and significant changes in HDAC expression by Min-U-Sil® 5 and no changes observed for NM-203; Min-U-Sil® 5 increased acetylated histone H3 lysine 4 (H3K4Ac), histone H3 lysine 9 (H3K9Ac) and acetylated histone H3 lysine 27 (H3K27Ac) on c-Myc promoter	[86]
SiO2-NP	Human population	DNA from leucocyte	Non exposed (n = 43, age: 37.95 ± 8.94) SiO2-NP exposed (n = 31, age: 35.52 ± 9.89, exposure duration: 2.49 ± 2.40 years)	Global DNA methylation (5-mC; HPLC)	No significant change in DNA methylation, increased oxidative stress	[54]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

Table 9.4 Summary table of studies reporting epigenetic changes induced by copper nanoparticles

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
CuO, 58.7 nm; specific surface area 16.2 m^2/g	In vitro	Human monocyte (THP1), primary small airway epithelial cells (SAEC), murine macrophage cells (RAW264.7)	The cells were in the respective cell culture media at two doses (0.5 and 30 μg/mL) for 24 h	Global DNA methylation (5-mC) and hydroxymethylation (5-hmC), LINE1 and Alu/ SINE methylation specific PCR	No significant difference in global DNA methylation; small hypermethylation observed in 5′-UTR, ORF1 and ORF2 of SAEC, ORF2 of THP-1	[56]
Green synthesized CuO NP; FE-SEM- 40 nm; DLS- 577 nm; zeta potential – 25.4 mV	In vitro	Human lung adenocarcinoma cell line (A549)		HDACs mRNA expression	Inhibition against total HDAC and different classes of HDACs.	[47]
CuO NPs (generated by using the thermal decomposition of metal organic precursor copper (II) acetylacetonate); 7.64–229.6 nm (inhalation chamber)	In vivo	Female ICR mice (8 animals/ group)	Whole-body inhalation chamber to 8×10^{-5} CuO NP/cm3 (geometric mean diameter 29.4 nm) for 3 days, 2 weeks, 6 weeks, and 3 months	Lung: Global DNA methylation (5-mC); miRNA expression; whole genome transcriptome analysis	No significant effect on global DNA methylation; mRNA expression changes (cell adhesion, migration, and cytokine production)	[83]
CuNP, 40–60 nm; purity 99.9%, spherical; specific surface area 12 m^2/g, bulk density 0.2 g/cm3	In vivo	Male albino Wistar rats (Han IGS rat [Crl:WI(Han)]), 5 weeks, body weight- 135 ± 10 g; housed randomly and individually in stainless steel cages at a stable temperature (21–22 °C), relative humidity 50% ± 10%, a 12-hr light-dark cycle, and a ventilation rate of 20 air changes per hour;	Five experimental groups: CuD (control without cu in mineral mixture): Diet with MX without cu (n = 8); CuS-H: Diet containing 6.5 mg/kg cu from CuCO3 (n = 8); CuS-L: Diet containing 3.25 mg/kg cu from CuCO3 (n = 8); CuNP-H: Diet containing 6.5 mg/kg cu from preparation of cu nanoparticles (n = 8); CuNP-L: Diet containing 3.25 mg/kg cu from preparation of cu nanoparticles (n = 8)	Global DNA methylation, oxidative stress markers	Cu supplementation reduces protein oxidation, nitration, DNA oxidation and methylation	[67]

(continued)

Table 9.4 (continued)

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
CuO, 58.7 nm; specific surface area 16.2 m^2/g	In vivo	Eight-week-old BALB/c male mice, mice housed environmentally controlled room at 25 ± 1 °C with a relative humidity of 50 ± 10% under a 12-h light/dark cycle, food and water were provided ad libitum;	CuO at 2.5 mg/kg body weight; Intratracheally instilled with these treatments and sacrificed 24 h after administration with an intraperitoneal injection of fatal-plus; macrophages, lung tissue and blood collected	Global DNA methylation (5-mC) and hydroxymethylation (5-hmC), LINE1 and Alu/SINE methylation specific PCR; expression of *DNMT1*, *DNMT3a* and *DNMT3b*	Increase in 5-mC levels and 5-hmC levels in lung tissue, methylation of LINE-1 not changed, SINE B1 elements hypermethylated in the alveolar macrophages	[55]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

9.2.5 Silver Nanoparticles

While silver nanoparticles are one of the most studied, both for its biomedical application and to identify its toxicity, the studies reporting epigenetic alterations are limited (Table 9.5). Gonzalez-Palomo et al.[37] in a study on EA.hy926 endothelial cells, observed global hypermethylation, downregulation of some miRNAs (miRNA -126, −155, and − 146) and increase in some inflammatory markers and levels of vascular cell adhesion molecule 1 (*VCAM-1*). Very few changes in DNA methylation were observed in BEAS-2B cells, exposed for a period of up to 6 weeks to Ag-NP (1 μg/mL), while significant changes in the transcriptome was observed [35]. Brzóska et al. [9] reported changes in miRNA expression (miR-499a, miR-1-3p), however DNA methylation were not altered in HepG2 cells, exposed to Ag-NP (20 nm, 10 μg/mL) for 24 h. In mouse hippocampal neuronal cell line (HT22), Mytych et al. [64] observed a significant increase in DNMT activity and global DNA methylation after 48 h of Ag-NP exposure and also after a recovery period of 96 h. In another study the authors observed significant H3 deacetylation and global DNA hypermethylation in A549 cells, exposed to higher concentration of PVP-coated Ag-NP [7]. While only a small percentage of PVP was part of particle preparation (99.9% Ag, 0.3% PVP), like in most epigenetic studies the influence of surface coating was not very clear [7]. Another study addressed the effect of Ag-NP on phosphorylation of histone H3 [103] in different cell lines (HaCaT, A549, MCF-7), where significant p-H3S10 (phosphorylation of histone H3 at serine 10) was observed, and was associated with cellular uptake of the particles. In contrast to other metal (oxide) nanoparticles, most studies on Ag-NP reported DNA hypermethylation, either at a global or sequence specific levels. However, the mechanistic explanation behind such changes have not been reported. Additionally, toxicologically relevant properties for silver nanoparticles such as effect of shape/size and ionization have also not been reported.

9.2.6 Gold Nanoparticles

Among metal nanoparticles, gold (Au-NP) has been studied rather extensively for epigenetic mechanism (Table 9.6), however with a focus, primarily on therapeutic properties for biomedical application. Majority of the particles used in such studies are biosynthesized, and are surface coated with citrate, PLGA etc. for specific application. In this section, we try to discuss the evidence on such epigenetic changes, focusing on the toxicological aspect where possible. However, it would be important to remember that surface coating of particle has an influence on uptake and thereby on the observed effects. Therefore, these results cannot be generalized for Au-NP as a group. Patil et al. [78] reported DNA methylation changes in human normal skin fibroblast (hypermethylation after 24 h, hypomethylation after 48 h) and A375 cells (hypomethylation after 24 and 48 h) and aberrant DNMT expression (*DNMT1*, *DNMT3A* and *DNMT3B*) exposed to biosynthesized Au-NP [78]. In another study the authors reported the reduction of cell cycle related protein expression and histone deacetylase activity in HepG2 cells treated with "gold-quercetin loaded into poly(DL-lactide-co-glycolide)" nanoparticles, however due to presence of multiple chemicals [6] such effect cannot be directly attributed to Au-NP. In another in vitro study, in human embryonic stem cells (hESCs), the authors investigated several cyto-genotoxicity and epigenetic endpoints for coated Au-NPs of different size (mercaptosuccinic acid coated-1.5, 4 nm, and citrate coated 14 nm) [87]. These nanoparticles exhibited global DNA hypomethylation (5-mC) and an increase in DNA hydroxymethylation (5-hmC) at sublethal concentrations. This effect was most prominent for Au-NP (4 nm) with 31,000 CpG sites showing demethylation [87]. However, no further interpretation on pathways were provided. In HepG2 cells, exposed to citrate coated Au-NP (10 μg/mL) for 24 h, Brzóska et al. reported changes in miRNA expression (miR-499a, miR-491-5p etc) however DNA methylation were not altered [9]. In a separate study, Ng et al. [65] reported that Au-Np (21 nm, citrate reduction) downregulated

Table 9.5 Summary table of studies reporting epigenetic changes induced by Silver nanoparticles

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
Ag-NP (<100 nm; sigma–Aldrich, cat. No. 758329)	In vitro	Mouse hippocampal neuronal cell line (HT22)	Freshly prepared stock solution of Ag-NPs (10 mg/ml in sterile PBS); 1 to 20 μg/ml, 48 h and 144 h (96 h after Ag-NP removal)	Global DNA methylation (5-mC); *DNMT1*, *DNMT3A* and *DNMT3B* expression	Global DNA hypermethylation and increase in DNMT expression	[64]
Ag-NP (1.8 ± 0.3 nm in diameter)	In vitro	Human endothelial cells (EA.hy926)	Ag-NP (0.1, 0.5, 1 μM), 24 h	Global DNA methylation (5-mC, flow cytometry- immunolabelling)	Global DNA hypermethylation	[37]
Ag-NP (10, 75 nm)	In vitro	Human bronchial epithelial cells (BEAS-2B cells)	1 μg/mL; cells were split (trypsinization), counted, re-seeded twice a week and re-exposed to the Ag-NPs for a total of 6 weeks.	Genome wide DNA methylation (Infinium human Methylation450 bead chip) and RNA-sequencing (RNA-Seq)	Small effect on DNA methylation, significant changes in transcriptome of 1717 genes, some of which were associated with fibrosis, epithelial-mesenchymal transition	[35]
Polyvinyl pyrrolidone (PVP)-coated Ag-NPs (99.9% Ag, 0.3% PVP, 20 nm; NanoAmor, Houston, USA)	In vitro	Human lung adenocarcinoma cell line (A549)	0–200 μg/mL of Ag-NPs for 48 and 72 h	Global DNA methylation (5-mC, ELISA), H3 acetylation (Western blot)	Significant deacetylation of H3, global DNA hypermethylation	[7]
Ag-NP, 20 nm, Plasmachem GmbH, Berlin, Germany	In vitro	Human hepatocyte carcinoma (HepG2)	10 μg/mL, 24 h	Gene specific methylation-EpiTect methylation array and miScript miRNA PCR Array- Human Inflammatory Response & Autoimmunity Signature Panel and human apoptosis signature panel	miRNA expression (miR-499a, miR-1-3p etc) significantly altered, no change in DNA methylation	[9]
Ag-NP (<100 nm; Sigma–Aldrich, cat. No. 576832, St. Louis, MO, USA)	In vitro	Human skin keratinocytes (HaCaT), human lung adenocarcinoma cell line (A549), human lung adenocarcinoma cell line (MCF-7)	0.1, 0.3, 0.5 and 1 mg/mL for 24 h	p-H3S10- phosphorylation of histone H3 at serine 10 (Western blot)	Increase in p-H3S10, in all 3 cell lines, and associated with uptake	[103]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

Table 9.6 Summary table of studies reporting epigenetic changes induced by Gold nanoparticles

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
Au-NP synthesized by Pseudoalteromonas, 23–30 nm; aliphatic compounds, aromatic ring and aromatic phosphates identified on surface	In vitro	Human normal skin fibroblast cell line, human skin melanoma cancer (A375)	AuNP concentrations (1000, 500, 250, 125, 62.5, 31.25, 15.625 μg/mL), 24 and 48 h	Immunochemical staining of DNA methylation (5-mC); DNMT expression (*DNMT1*, *DNMT3a*, *DNMT3b*)	Human normal skin fibroblast: Hypermethylation after 24 h, hypomethylation after 48 h; A375 cells: Hypomethylation after 24 and 48 h; aberrant DNMT expression (DNMT1, DNMT3A and DNMT3B)	[78]
Au-Np (1.5, 4, and 14 nm); Au-NP (1.05 nm and 4 nm) capped with mercaptosuccinic acid (MSA); Au-NP (14 nm) citrate coated	In vitro	Human embryonic stem cells (hESCs)	Sublethal dosage Au-NP 1.5 nm (1 μg mL − 1), Au-NP 4 nm (10 μg mL − 1), Au-NP 14 nm (10 μg mL − 1); 24 h	5-mC immunoprecipitation-based colorimetric assay; HM450K array	Global hypomethylation for all particles, highest for Au-NP 4 nm; increase in hydroxymethyl cytosine (5-hmC), DNA hypomethylations in 31,000 CpG sites for Au-NP 4 nm	[87]
Au-NP, 20 nm; synthesized by citrate reduction of gold salts	In vitro	Human fetal fibroblasts (MRC5), primary small airway epithelial cells (SAEC)	1 nM, 48 and 72 h	Bisulfite sequencing of PROS1	No change in DNA methylation of the PROS1	[65]
Au-NP (20 nm), NanoComposix, San Diego, CA, USA; Sodium-citrate-coated	In vitro	Human hepatocyte carcinoma (HepG2)	10 μg/mL, 24 h	Gene specific methylation-EpiTect methylation array and miScript miRNA PCR Array-Human Inflammatory Response & Autoimmunity Signature Panel and human apoptosis signature panel	miRNA expression (miR-499a, miR-491-5p etc) significantly altered, no change in DNA methylation	[9]
Au-NP (5, 60 and 250 nm); Citrate-coated colloidal AuNPs	In vivo	Male BALB/c mice (~20 g, 7 weeks old), housed in a conventional animal house at controlled temperature (21 ± 1 °C) and humidity (50 ± 10%) with 12-h dark/light cycles	Vehicle control (n = 8); AuNPs (n = 5/group): 5 nm, low dose (0.25 mg/kg) and high dose (2.5 mg/kg); 60 nm, low dose (0.25 mg/kg) and high dose (2.5 mg/kg); 250 nm, low dose (0.25 mg/kg) and high dose (2.5 mg/kg); by single intra-tracheal instillation, 48-h post exposure animal sacrificed	Global DNA methylation and hydroxymethylation (LC–MS/MS), sequence specific DNA methylation	No changes in global methylation (5-mC) and hydroxymethylation (5-hmC) levels in mouse lungs; AuNP 60 nm induced CpG hypermethylation in *ATM*, *CDK* and *GSR* genes and hypomethylation in *GPX*; *GSR* and *Trp53*	[94]

(continued)

Table 9.6 (continued)

Nanoparticle properties	Experiment[a]			Epigenetic (and associated) endpoint studied[b]	Result	References
	Type	System	Condition			
Au-NP, colloidal suspension (40 and 100 nm), BB international (Cardiff, UK)	In vivo	Female Swiss albino mice (H strain), weighing 28–30 g and aged 2.5–3 months; mated with males of the same cross, mating ratio- 1:3	Pregnant mice divided into 3 groups-untreated controls (2 dams); au-NP, 40 nm (3 dams); and au-NP, 100 nm (2 dams). Additional animals used for clastogenicty study. Pregnant mice were treated on days 10, 12, 14 and 17 of gestation with 1 ml/mouse of AuNP suspensions (either 40 nm or 100 nm), injected intraperitoneally (i.p), delivering an average dose of 3.3 mg/kg body weight.	MiRNAs (miRCURY LNA microRNA Array, seventh generation) were evaluated in the lungs and livers of fetuses on day 18 of pregnancy	Changes associated with cell proliferation, apoptosis, inflammation	[3]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

the expression of PROS1 (Vitamin K-dependent protein S) gene, mediated by the up-regulation of microRNA-155 (miR-155) but no change in methylation was observed for PROS1.

From the limited in vivo evidence, it is also clear that Au-NPs can induce epigenetic alterations in different tissue/cell types. In a study by our group [94], in BALB/c mice exposed to different sizes of citrate coated Au-NP (5, 60, 250 nm), while no changes were observed on the level of global DNA methylation (5-mC) and hydroxymethylation (5-hmC), Au-NP (60 nm) induced CpG hypermethylation in *ATM*, *CDK* and *GSR* genes and hypomethylation in *GPX*, *GSR* and *TRP53*. These changes were also associated with deregulations in immune markers. In another study, transplacental miRNA expression changes associated with cell proliferation, apoptosis, inflammation etc. were also observed in liver and lung of mice foetus, for Au-NP (40, 100 nm) administered intraperitonially (3.3 mg/Kg) [3].

9.3 Epigenetic Changes Induced by Carbon Nanotubes

Due to the growing evidence of toxicity induced by carbon nanotubes (CNTs), and the increasing emphasis on high aspect ratio, and the asbestos analogy [49, 81], there has been considerable research on epigenetic changes compared to other NPs. Classification of “MWCNT-7” as “Possibly carcinogenic to humans” (International Agency for Research on Cancer- IARC Group 2B) [43] and growing emphasis on mechanistic evidence for potential carcinogenicity, has also highlighted the need of epigenetic study. In this section we discuss the studies (Table 9.7) that have used different types of CNTs (MWCNT-Multi wall, SWCNT- Single wall) to evaluate epigenetic alterations, and where possible try to draw parallels with that of evidence available for asbestos.

From our study, in human bronchial epithelial cells (16HBE), we observed significant changes in DNA methylation induced by MWCNTs (JRC NM400; diameter 11 nm, length 846 nm) and SWCNTs (NIST SRM:2483; diameter 0.8 nm, length 8000 nm) [69]. While we did not observe global DNA methylation changes [33, 69] and sequence specific changes in LINE1 methylation [33], we observed significant changes in individual CpG sites for MWCNT (2398 genes were hypomethylated at gene promoters), and SWCNTs (589 CpG sites hypo- or hypermethylated), associated with pathways such as p53 signaling, DNA damage repair and cell cycle. We also were able to identify sequence specific changes in DNA methylation of several important genes such as *NPAT/ATM*, *PIK3R2*, *DNMT1*, *HDAC4*, *SKI* and *GSTP1* [33, 69]. We did not observe significant changes in miRNA expression, which could however be due to the smaller number of replicates [33]. In a subsequent study we compared the epigenetic alterations observed for CNTs to that induced by asbestos in 16 HBE cells (Esra [24]), and mostly observed no significant changes in global DNA, however sequence specific methylation was altered for *ATM* (chrysotile, SWCNT, and MWCNT), *CDKN1A* (SWCNT, MWCNT, and amosite) and *TRAF2* (only SWCNT) (Esra [24]) all of which play important role in pathways associated with DNA damage and apoptosis. In our study we also did not observed significant changes in global RNA methylation, which is a post-a translational modification with important role in gene regulatory processes. Our analysis of genome wide methylation changes induced by asbestos fibers (amosite, crocidolite and chrysotile) in 16HBE cells [70] also revealed significant changes in methylation pattern (associated with MAPK signaling pathway, pathways in cancer, WNT gene family and homeobox group) and some of the altered pathways were similar to that observed for CNTs. Differentially methylated regions including HOX genes have also been reported by Kettunen et al. [51] for asbestos-exposed patients. In a recent study, we reported the effect of long-term CNT and amosite exposure and recovery on DNA methylation, and we found that SWCNTs/amosite induced hypermethylation at CpG sites and MWCNT induced hypomethylation at CpG sites [71]. Moreover, spontaneous DNA methylation changes were observed during the recovery period [71].

Table 9.7 Summary table of studies reporting epigenetic changes induced by carbon nanotubes

	Experiment[a]					
Nanoparticle properties	Type	System	Condition	Epigenetic (and associated) endpoint studied[b]	Result	References
MWCNT (NM-400, JRC)-diameters 11 nm, length 846 nm; SWCNT (NISTS, RM:2483)-diameters 0.8 nm, length 8000 nm	In vitro	Human Bronchial Epithelial cell line (16HBE)	25 and 100 μg/ml, 24 h	Global DNA methylation (5-mC; LC-MS/MS); genome-wide (Infinium HumanMethylation450 BeadChip Array); gene-specific DNA methylation (pyrosequencing) and RNA-expression analyses (RNA sequencing)	No global DNA methylation alteration on 5-methylcytosine (5-mC); MWCNTs: 2398 genes were hypomethylated (at gene promoters), SWCNTs: 589 CpG sites (located on 501 genes) were either hypo- (N = 493 CpG sites) or hypermethylated (N = 96 CpG sites); SWCNT exposure showed hypermethylation of SKI proto-oncogene (*SKI*), glutathione S-transferase pi 1 (*GTSP1*), SHROOM family member 2 (*SHROOM2*)	[69]
MWCNT (NM-400, JRC)-diameters 11 nm, length 846 nm; SWCNT (NISTS, RM:2483)-diameters 0.8 nm, length 8000 nm	In vitro	Human Bronchial Epithelial cell line (16HBE)	2.5, 5 and 25 μg/ml, 24 h	Global DNA methylation (5-mC; LC-MS/MS); gene-specific DNA methylation (pyrosequencing) and miRNA-expression analyses (TaqMan® Array human MicroRNA)	No global DNA methylation alteration on 5-methylcytosine (5-mC) or LINE 1 elements; sequence specific changes in *DNMT1, HDAC4, MAP3K10, NPAT/ATM* genes; no significant changes in miRNA expression	[33]

MWCNT (NM-400, JRC)-diameters 11 nm, length 846 nm; SWCNT (NISTS, RM:2483)-diameters 0.8 nm, length 8000 nm	In vitro	Human monocyte (THP1)	MWCNT/SWCNT (25 and 100 µg/ml); 24 h	Global DNA methylation (5-mC; LC-MS/MS); genome-wide (Infinium HumanMethylation450 BeadChip Array)	No global methylation or hydroxymethylation, significant gene specific methylation, collectively CNTs induced gene promoter-specific hypomethylation in 1127 different genes- associated with platelet activation, VEGF receptor signaling pathway, chromatin organization, pathways in cancer, MAPK signaling pathway, PI3K-Akt signaling pathway	[68]
MWCNT (NM-400, JRC)-diameters 11 nm, length 846 nm; SWCNT (NISTS, RM:2483)-diameters 0.8 nm, length 8000 nm; Asbestos- Amosite (South African, NB #4173–111-4), Crocidolite (South African, NB #4173–111-3) and Chrysotile (“A” Rhodesian, NB #4173–111-2)	In vitro	Human Bronchial Epithelial cell line (16HBE)	MWCNT/ SWCNT (25 and 100 µg/ml), Asbestos (2.5 µg/ml); 24 h	Global DNA and RNA (hydroxy)methylation (LC-MS/MS); m6A-RNA methylation (ELISA); gene-specific DNA methylation (pyrosequencing)	MWCNT-significant global DNA hypomethylation of cytosine and global RNA hypomethylation of adenosine; ATM methylation was affected by chrysotile, SWCNT, and MWCNT; decreased DNA methylation of CDKN1A for SWCNT, MWCNT, and amosite.	[24]
MWCNT (NM-400, JRC)-diameters 11 nm, length 846 nm; SWCNT (NISTS, RM:2483)-diameters 0.8 nm, length 8000 nm; Asbestos- Amosite (South African, NB #4173–111-4)	In vitro	Human Bronchial Epithelial cell line (16HBE)	MWCNT/ SWCNT (0.25 µg/ml), Asbestos (0.05 µg/ml); total of 4 weeks +2 weeks of recovery	Genome-wide (Infinium MethylationEPIC BeadChip Kit-EPIC array)	Hippocalcinlike 1 (*HPCAL1*), protease serine 3 (*PRSS3*), kallikrein-related peptidase 3 (*KLK3*), kruppel like factor 3 (*KLF3*) genes were hypermethylated at different time points in either SWCNT-exposed or amosite-exposed cells. Spontaneous DNA methylation alterations were noted even after the recovery period.	[71]

(continued)

Table 9.7 (continued)

MWCNT (Cheaptubes Inc., Cambridgeport, VT): LW- long length and wide diameter (length: 10–30 μm, diameter: 20–30 nm), LN- long length and narrow diameter (length: 10–30 μm, diameter: 8–15 nm), SN- short length and narrow diameter (length: 0.5–2.0 μm, diameter: 8–15 nm)	In vitro	Human bronchial epithelial cells (BEAS-2B cells), human hepatocyte carcinoma (HepG2)	EC20 (mg/L)- HepG2 cells (LW- 45.28, LN- 38.52, SN- 8.37), BEAS-2B (LW- 25.19, LN- 9.68, SN- 6.34); 24 h	Global DNA methylation (5-mC); DNMT1, DNMT3A and DNMT3B activity	Global DNA hypermethylation in HepG2 cells, global DNA hypomethylation in BEAS-2B cells	[15]
MWCNT (NM401; length- 6012.09 ± 4091.45 NM, diameter- 64.2 ± 34.5 nm)	In vitro	Human bronchial epithelial cells (BEAS-2B cells)	10 and 20 mg/mL for up to 4 weeks; exposure media changed every 3 days, sample collected at 2 ad 4 weeks	Genome wide methylation- human methylation450 BeadChip	DNA methylation changes of 755 CpG sites- corresponding to 453 genes; mostly hypomethylated	[90]
Rigid MWCNT (Mitsui, diameter-22 nm; length- 13,000 nm)	In vitro	Human monocyte (THP1)	THP1 were and differentiated for 48 h with 50 nM PMA; 5, 10, and 20 μg of rCNT/mL, for 24, 48 or 72 h	Genome wide methylation- human methylation BeadChip	Significant changes in DNA methylation, and transcriptomic changes in pathways associated with cell cycle regulation, immune system, signal transduction, metabolism	[85]
MWCNTs (Cheaptubes Inc., Cambridgeport, VT) NS- narrow diameter and short length (10–20 nm, 0.5–2 μm), WS- wide diameter and short length (30–50 nm, 0.5–2 μm), NL- narrow diameter and long length (10–20 nm, 10–30 μm)	In vivo	Male and female C57BL/6 mice (2 month), maintained in specific pathogen-free conditions (22 ± 2 °C, 30–40% humidity, 12 h light/12 h dark cycles)	Mice were exposed to MWCNTs by oropharyngeal aspiration; after 24 h or 7 days, mice were euthanized; lung lavage fluid and lung tissues were harvested	Luminometric methylation assay and sequence specific methylation (pyrosequencing)- IL-1ß, IL-6, and TNF-α	Significant global hypomethylation (NL > WS > NS); significant sequence specific promoter hypomethylation of *IL-1β, IL-6*, and *TNF-α* at 24 h followed by a reversal of the trend at 7 days post exposure	[18]

MWCNT (NM-400, JRC)-diameters 11 nm, length 846 nm; SWCNT (NISTS, RM:2483)-diameters 0.8 nm, length 8000 nm	In vivo	Male BALB/c mice (~20 g, 7 weeks old), housed in a conventional animal house at controlled temperature (21 ± 1 °C) and humidity (50 ± 10%) with 12-h dark/light cycles	Vehicle control (n = 8); CNTs (n = 5/group); single-walled CNTs (SWCNTs), low dose (0.25 mg/kg) and high dose (2.5 mg/kg) and multi-walled CNTs (MWCNTs) low dose (0.25 mg/kg) and high dose (2.5 mg/kg); by single intra-tracheal instillation, 48-h post exposure animal sacrificed	Global DNA methylation and hydroxymethylation (LC–MS/MS), sequence specific DNA methylation	No changes in global methylation (5-mC) and hydroxymethylation (5-hmC) levels in mouse lungs; SWCNTs and MWCNTs had shape effects on promoter methylation of ATM	[94]
CNT (long and short), amosite (long and short), characterization reported elsewhere.	In vivo	C57BL/6 strain mice (eight-week-old female, Charles River laboratories, UK), maintained on a normal 12 hr. light and dark cycle.	Intrapleural injection; vehicle control (VC), SFA, LFA, SNT or LNT. For 1- and 12-weeks exposure, CNT (5 mg/mouse, n = 4). For 6 months and 1-year exposure: VC, LFA and LNT, with n = 4, dose of 2.5 mg/mouse (100 mL) of LNT. For prolonged (up to 20 months) exposure, mice were injected with 1 mg/mouse of LNT (n = 4), 0.5 mg/mouse of LNT (n = 5) or 0.2 mg/mouse of LNT (n = 12). Asbestos fiber samples were administered at a dose of 25 mg/mouse (100 mL) (n = 4) for short-term studies and n = 16, prolonged exposure) or at 50 mg/mouse (n = 16, prolonged exposure).	Bisulphite sequencing	Hypermethylation of p16/Ink4a and p19/Arf for long CNT and Asbestos, preceded mesothelioma induction	[16]

(continued)

Table 9.7 (continued)

MWCNT (200 nm–100 μm agglomerates)	Human population	DNA extracted from whole blood	Control (n = 43, age: 34.64 ± 8.57), exposed (n = 24, age: 35.87 ± 6.90, average duration of exposure: 4.25 ± 2.40 years); based on levels of EC in workplace was determined to be between 4.6 and 42.6 μg/m3- three categories of exposed workers: 'Lab-low' (n = 9; 1 μg/m3 EC), 'lab-high' (n = 6; 7 μg/m3 EC), and 'operators' (n = 7; 45 μg/m3 EC);	Global DNA methylation (5-mC, 5-hmC; LC-MS/MS); gene-specific DNA methylation (pyrosequencing)	No significant difference in global methylation (5-mC), hydroxymethylation (5-hmC) and LINE1 methylation; significant change in methylation for *DNMT1, ATM, SKI*, and *HDAC4* promoter CpGs in MWCNT-exposed workers	[31]
MWCNT	Human population	RNA was extracted from whole blood	Control (n = 7, 20–30 years), MWCNT (n = 8, 23–60 years, 6–24 months working, average inhalable EC concentration: 14.42 ± 3.8 μg/m3)	miRNA, ncRNA and mRNA expression	529 lncRNAs were upregulated and 448 lncRNAs were downregulated; A total of 29 miRNAs, 2404 lncRNAs and 2655 mRNAs dysregulated by high MWCNT exposure; inflammation, fibrosis, tumor progression and cancer	[89]

[a]Limited exposure information provided here, for additional information refer to full text
[b]For other endpoints refer to full text

However, the induction of DNA methylation changes for the chronic exposure period was less pronounced compared to our acute exposure experiment and such difference could be due to both the lower concentration of exposure for the chronic phase and possibly due to tolerance and adaptation. While in our study we observed fewer DNA methylation changes, in a long-term study in BEAS-2B cells, with MWCNT (NM401) exposure period of up to 4 weeks, Sierra et al. [90] observed a significant hypomethylation of 697 CpG, primarily associated with functions such as cell adhesion. The difference between the two long terms studies could primarily be due to the difference in lower exposure concentration (0.25 μg/ml) used in our study [71] compared to that reported by Sierra et al. [90] (20 μg/ml), but also due to the difference in cell lines used.

From one of our study, in BALB/c mice, intratracheal administration of MWCNT and SWCNT resulted in a sequence specific DNA methylation in ATM [94]. Such changes have been observed in other cell types as well. For instance, in one of our studies in THP1 monocytes, while we observed no changes in global methylation or hydroxymethylation, significant gene specific methylation associated with pathways such as platelet activation, VEGF receptor signaling pathway, chromatin organization, pathways in cancer, MAPK signaling pathway, PI3K-Akt signaling pathway were observed [68]. In another study, Saarimäki et al. [85] reported significant changes in transcriptome and epigenome (DNA methylation) associated with pathways related to immune system, cell cycle and signal transduction, in differentiated THP1 monocyte exposed to rigid MWCNT.

In an in vitro study, Chatterjee et al. [15] critically evaluated the effect of MWCNT diameter and length in BEAS-2B and HepG2 cells. In the study, the authors observed a global DNA hypermethylation in HepG2 cells while a global DNA hypomethylation was observed for BEAS-2B cells [15]. These changes were in line with DNMT1 and DNMT3B expression in both cell lines. Of the different forms of MWCNT studied, long-narrow and short-narrow forms induced the most significant epigenetic changes, associated with other oxidative stress and inflammatory markers. In another study, significant global hypomethylation was observed in lung tissue of C57BL/6 mice, exposed to MWCNTs of different length and diameter by oropharyngeal aspiration [18]. In the study narrow diameter and long length MWCNT induced the most significant effect [18], in line with the observation made by Chatterjee et al. [15]. The authors also reported significant sequence specific promoter hypomethylation of IL-1ß, IL-6, and TNF-α at 24 h, associated with increase in the inflammatory markers, followed by a reversal of the trend 7 days post exposure [18]. These two studies [15, 18] taken together, provide significant evidence regarding the influence of diameter and aspect ratio on CNT induced toxicity and epigenotoxicity. Another evidence in support of the asbestos analogy and high aspect ratio fiber toxicity comes from the in vivo study of Chernova et al. [16], where the authors used long and short forms of Amosite and carbon nanotubes. In the study the authors observed induction of mesothelioma, by pleural injection of long CNT and long amosite asbestos fibers, preceded by hypermethylation of overlapping genes p16/Ink4a and p19/Arf [16], which are often inactivated in different forms of cancer.

In addition, studies have identified considerable evidence of occupational exposure to CNTs, and has been presented in a recent review by Canu et al. [38]. In a study by our group [31], while we did not observe any significant difference in global methylation (5-mC), hydroxymethylation (5-hmC) and LINE1 methylation; we observed significant changes in CpG methylation for *DNMT1*, *ATM*, *SKI*, and *HDAC4* in MWCNT exposed workers. These sequence specific methylation changes were also observed in our in vitro studies as discussed above. In another cross-sectional study, Shvedova et al. [89] observed significant enrichment of miRNA-lncRNA-mRNA changes in inflammation, fibrosis, tumor promotion and cancer progression pathways in MWCNT exposed workers. While these studies do not allow for a definitive inter-

pretation regarding the cause or dose response relationship, some other cross-sectional studies have shown significant changes in inflammation and effect on cardiovascular and lung health [25, 34, 98]. These studies provided the first set of evidence of epigenetic alterations induced by occupational exposure to CNTs, but larger longitudinal population studies are needed to provide more detailed inside.

9.4 Summary and Perspectives

From the studies presented above, it is clear that NPs can induce epigenetic changes both at toxic and sub- cytotoxic concentrations. In the studies discussed above, with the exception of Ag-NP, most of the particles induced global DNA hypomethylation. DNA hypomethylation is observed in carcinogenesis and is highly correlated with methylation of DNA repeat elements [22]. Methylation of repeat elements such as LINE 1 and Alu represent approximately 50% of global methylation levels and hypomethylation has been associated with genomic instability and therefore can be used to study epigenetic alterations induced by NPs. Additionally, it has been established that global DNA methylation levels are modulated by oxidative stress[61, 73], and these mechanisms share one-carbon metabolism as the common denominator. This was observed for several of the NPs, but mostly for TiO2-NP, as discussed above. In a relatively small number of studies and primarily in case of Ag-NP DNA hypermethylation has been observed as well. DNA hypermethylation at global level and at specific loci has been implicated in large number of diseases including cancer [23]. Sequence specific DNA hypermethylation of *GSTP1*, *TP53*, *p16*, *p21*, *ATM* among other important genes, increased phosphorylation of H3 and concurrent mRNA dysregulation has been observed for the nanoparticles, similar to that observed for other genotoxic carcinogens such as formaldehyde, benzene [14]. While these findings are extremely interesting, it limits further mechanistic interpretation due to certain study limitations discussed further. While most epigenetic changes have been observed at a sub cytotoxic concentration, the observed results are representative of a single time window. Given that epigenetic changes are highly dynamic, and most of the nanoparticles were studied for shorter duration of exposure, the results cannot provide information of a chronic exposure scenario or that resulting from a change in exposure conditions. Therefore, to understand the dynamic nature of such changes, studies need to be designed taking into account- **chronic low exposure**, different time windows and an inclusion of recovery period. Another important aspect of a study design, aimed at understanding epigenetic effect should be the selection of appropriate **cell type**, representative of the exposure and elimination route. Despite the limited evidence, this is something that has been observed for NPs [80] and is extremely important as epigenetic changes are cell type specific. Additional experimental conditions, relevant for nanotoxicological experiments in general, such as **physico-chemical properties**, dispersion condition, shape of material, aspect ratio of fibres, surface coating and functionalization are equally important in the interpretation of epigenetic changes. Furthermore, these changes observed in mostly in vitro studies must be validated in vivo and if possible, in exposed human population. Epigenetic alterations can be valuable in providing key mechanistic insight which can play important role in risk assessment, regulation, and classification based on mechanistic evidences by agencies such as the IARC [17, 92].

References

1. Asweto CO et al (2017) Cellular pathways involved in silica nanoparticles induced apoptosis: a systematic review of in vitro studies. Environ Toxicol Pharmacol:191–197. https://doi.org/10.1016/j.etap.2017.09.012
2. Bai W, Chen Y, Gao A (2015) Cross talk between poly(ADP-ribose) polymerase 1 methylation and oxidative stress involved in the toxic effect of anatase titanium dioxide nanoparticles. Int J Nanomedicine 10:5561–5569. https://doi.org/10.2147/IJN.S88059
3. Balansky R et al (2013) Transplacental clastogenic and epigenetic effects of gold nanoparticles in mice. Mutat Res 751–752(1):42–48. https://doi.org/10.1016/j.mrfmmm.2013.08.006

4. Baylin SB (2005) DNA methylation and gene silencing in cancer. Nat Clin Pract Oncol:S4–S11. https://doi.org/10.1038/ncponc0354
5. Baylin SB et al (2001) Aberrant patterns of DNA methylation, chromatin formation and gene expression in cancer. Hum Mol Genet 10(7):687–692. https://doi.org/10.1093/hmg/10.7.687
6. Bishayee K, Khuda-Bukhsh AR, Huh SO (2015) PLGA-loaded gold-nanoparticles precipitated with quercetin downregulate HDAC-Akt activities controlling proliferation and activate p53-ROS crosstalk to induce apoptosis in hepatocarcinoma cells. Mol Cells 38(6):518–528. https://doi.org/10.14348/molcells.2015.2339
7. Blanco J et al (2017) Polyvinyl pyrrolidone-coated silver nanoparticles in a human lung cancer cells: time- and dose-dependent influence over p53 and caspase-3 protein expression and epigenetic effects. Arch Toxicol 91(2):651–666. https://doi.org/10.1007/s00204-016-1773-0
8. Boccuni F et al (2020) Occupational exposure to graphene and silica nanoparticles. Part I: workplace measurements and samplings. Nanotoxicology 14(9):1280–1300. https://doi.org/10.1080/17435390.2020.1834634
9. Brzóska K, Grądzka I, Kruszewski M (2019) Silver, gold, and iron oxide nanoparticles Alter miRNA expression but do not affect DNA methylation in HepG2 cells. Materials 12(7):1038. https://doi.org/10.3390/ma12071038
10. Carmo TLL et al (2019) Overview of the toxic effects of titanium dioxide nanoparticles in blood, liver, muscles, and brain of a Neotropical detritivorous fish. Environ Toxicol 34(4): 457–468. https://doi.org/10.1002/tox.22699 LK - http://limo.libis.be/resolver?&sid=EMBASE&issn=15227278&id=doi:10.1002%2Ftox.22699&atitle=Overview+of+the+toxic+effects+of+titanium+dioxide+nanoparticles+in+blood%2C+liver%2C+muscles%2C+and+brain+of+a+Neotropical+detritivorous+fish&stitle=Environ.+Toxicol.&title=Environmental+Toxicology&volume=34&issue=4&spage=457&epage=468&aulast=Carmo&aufirst=Talita+L.+L.&auinit=T.L.L.&aufull=Carmo+T.L.L.&coden=ETOXF&isbn=&pages=457-468&date=2019&auinit1=T&auinitm=L.L
11. Carriere M et al (2017) Impact of nanoparticles on DNA repair processes: current knowledge and working hypotheses. Mutagenesis 32(1):203–213. https://doi.org/10.1093/mutage/gew052
12. Cedar H, Bergman Y (2009) Linking DNA methylation and histone modification: patterns and paradigms. Nat Rev Genet 10(5):295–304. https://doi.org/10.1038/nrg2540
13. Chakraborty B et al (2016) Immunomodulatory properties of silver nanoparticles contribute to anticancer strategy for murine fibrosarcoma. Cell Mol Immunol 13(2):191–205. https://doi.org/10.1038/cmi.2015.05
14. Chappell G et al (2016) Epigenetic alterations induced by genotoxic occupational and environmental human chemical carcinogens: a systematic literature review. Elsevier. https://doi.org/10.1016/j.mrrev.2016.03.004
15. Chatterjee N et al (2016) Diameter size and aspect ratio as critical determinants of uptake, stress response, global metabolomics and epigenetic alterations in multi-wall carbon nanotubes. Carbon 108:529–540
16. Chernova T et al (2017) Long-fiber carbon nanotubes replicate asbestos-induced mesothelioma with disruption of the tumor suppressor gene Cdkn2a (Ink4a/Arf). Curr Biol 27(21): 3302–3314.e6. https://doi.org/10.1016/j.cub.2017.09.007
17. Cogliano VJ et al (2008) Use of mechanistic data in IARC evaluations. Environ Mol Mutagen:100–109. https://doi.org/10.1002/em.20370
18. Cole E et al (2019) Multiwalled carbon nanotubes of varying size lead to dna methylation changes that correspond to lung inflammation and injury in a mouse model. Chem Res Toxicol 32(8):1545–1553. https://doi.org/10.1021/acs.chemrestox.9b00075
19. Definition of epigenetic alteration – NCI Dictionary of Cancer Terms – National Cancer Institute (n.d.). Available at: https://www.cancer.gov/publications/dictionaries/cancer-terms/def/epigenetic-alteration. Accessed 4 Feb 2021
20. Dhupal M et al (2018) Immunotoxicity of titanium dioxide nanoparticles via simultaneous induction of apoptosis and multiple toll-like receptors signaling through ROS-dependent SAPK/JNK and p38 MAPK activation. Int J Nanomedicine 13:6735–6750. https://doi.org/10.2147/IJN.S176087
21. Dinicola S et al (2015) Multiwalled carbon nanotube buckypaper induces cell cycle arrest and apoptosis in human leukemia cell lines through modulation of AKT and MAPK signaling pathways. Toxicol In Vitro 29(7):1298–1308. https://doi.org/10.1016/j.tiv.2015.05.006
22. Ehrlich M (2009) DNA hypomethylation in cancer cells. Epigenomics:239–259. https://doi.org/10.2217/EPI.09.33
23. Ehrlich M (2019) DNA hypermethylation in disease: mechanisms and clinical relevance. Epigenetics:1141–1163. https://doi.org/10.1080/15592294.2019.1638701
24. Emerce E et al (2019) Carbon nanotube- and Asbestos-induced DNA and RNA methylation changes in bronchial epithelial cells. Chem Res Toxicol 32(5). https://doi.org/10.1021/acs.chemrestox.8b00406
25. Fatkhutdinova LM et al (2016) Fibrosis biomarkers in workers exposed to MWCNTs. Toxicol Appl Pharmacol 299:125–131. https://doi.org/10.1016/j.taap.2016.02.016
26. Ferreira HJ, Esteller M (2018) Non-coding RNAs, epigenetics, and cancer: tying it all together. Cancer Metastasis Rev 37(1):55–73. https://doi.org/10.1007/s10555-017-9715-8
27. Fuks F (2005) DNA methylation and histone modifications: teaming up to silence genes. Curr Opin

Genet Dev:490–495. https://doi.org/10.1016/j.gde.2005.08.002

28. Gao F et al (2016) Zinc oxide nanoparticles-induced epigenetic change and G2/M arrest are associated with apoptosis in human epidermal keratinocytes. Int J Nanomedicine 11:3859–3874. https://doi.org/10.2147/IJN.S107021
29. Geiss O et al (2020) Characterisation of food grade titania with respect to nanoparticle content in pristine additives and in their related food products. Food Addit Contam Part A 37(2):239–253. https://doi.org/10.1080/19440049.2019.1695067
30. Ghosh M et al (2012) In vitro and in vivo genotoxicity of silver nanoparticles. Mutat Res 749(1–2). https://doi.org/10.1016/j.mrgentox.2012.08.007
31. Ghosh M, Öner D, Poels K, Tabish AM et al (2017a) Changes in DNA methylation induced by multi-walled carbon nanotube exposure in the workplace. Nanotoxicology 11(9–10). https://doi.org/10.1080/17435390.2017.1406169
32. Ghosh M, Öner D, Duca RC et al (2017b) Cyto-genotoxic and DNA methylation changes induced by different crystal phases of TiO2-np in bronchial epithelial (16-HBE) cells. Mutat Res 796:1–12. https://doi.org/10.1016/j.mrfmmm.2017.01.003
33. Ghosh M et al (2018) Single-walled and multi-walled carbon nanotubes induce sequence-specific epigenetic alterations in 16 HBE cells. Oncotarget 9(29):20351–20365. https://doi.org/10.18632/oncotarget.24866
34. Ghosh M et al (2020) Increased telomere length and mtDNA copy number induced by multi-walled carbon nanotube exposure in the workplace. J Hazard Mater 394:122569. https://doi.org/10.1016/j.jhazmat.2020.122569
35. Gliga AR et al (2018) RNA-sequencing reveals long-term effects of silver nanoparticles on human lung cells. Sci Rep 8(1):1–14. https://doi.org/10.1038/s41598-018-25085-5
36. Gong C et al (2012) Methylation of PARP-1 promoter involved in the regulation of nano-SiO 2-induced decrease of PARP-1 mRNA expression. Toxicol Lett 209(3):264–269. https://doi.org/10.1016/j.toxlet.2012.01.007
37. González-Palomo AK et al (2021) Effect of silver nanoparticles (AgNPs) exposure on microRNA expression and global DNA methylation in endothelial cells EA.hy926. Environ Toxicol Pharmacol 81:103543. https://doi.org/10.1016/j.etap.2020.103543
38. Guseva Canu I et al (2020) State of knowledge on the occupational exposure to carbon nanotube. Int J Hyg Environ Health 225:113472. https://doi.org/10.1016/j.ijheh.2020.113472
39. Halappanavar S et al (2011) Pulmonary response to surface-coated nanotitanium dioxide particles includes induction of acute phase response genes, inflammatory cascades, and changes in microRNAs: a toxicogenomic study. Environ Mol Mutagen:425–439. https://doi.org/10.1002/em.20639
40. Handy DE, Castro R, Loscalzo J (2011) Epigenetic modifications: basic mechanisms and role in cardiovascular disease. Circulation 123(19):2145–2156. https://doi.org/10.1161/CIRCULATIONAHA.110.956839
41. Hanly DJ, Esteller M, Berdasco M (2018) Interplay between long non-coding RNAs and epigenetic machinery: emerging targets in cancer? Philos Trans R Soc B Biol Sci. https://doi.org/10.1098/rstb.2017.0074
42. Huaux F et al (n.d.) Mesothelioma response to carbon nanotubes is associated with an early and selective accumulation of immunosuppressive monocytic cells. Part Fibre Toxicol 13(1):46. https://doi.org/10.1186/s12989-016-0158-0
43. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans (2017) Carbon nanotubes. IARC Monogr 111
44. Jayaram DT, Payne CK (2020a) Food-grade TiO2Particles generate intracellular superoxide and Alter epigenetic modifiers in human lung cells. Chem Res Toxicol 33(11):2872–2879. https://doi.org/10.1021/acs.chemrestox.0c00331
45. Jayaram DT, Payne CK (2020b) Intracellular generation of superoxide by TiO2Nanoparticles decreases histone deacetylase 9 (HDAC9), an epigenetic modifier. Bioconjug Chem 31(5):1354–1361. https://doi.org/10.1021/acs.bioconjchem.0c00091
46. Jin B, Robertson KD (2013) DNA methyltransferases, DNA damage repair, and cancer. Adv Exp Med Biol:3–29. https://doi.org/10.1007/978-1-4419-9967-2_1
47. Kalaiarasi A et al (2018) Copper oxide nanoparticles induce anticancer activity in A549 lung cancer cells by inhibition of histone deacetylase. Biotechnol Lett 40(2):249–256. https://doi.org/10.1007/s10529-017-2463-6
48. Kalishwaralal K et al (2009) Silver nanoparticles inhibit VEGF induced cell proliferation and migration in bovine retinal endothelial cells. Colloids Surf B Biointerfaces 73(1):51–57. https://doi.org/10.1016/j.colsurfb.2009.04.025
49. Kane AB, Hurt RH, Gao H (2018) The asbestos-carbon nanotube analogy: an update. Toxicol Appl Pharmacol 361:68–80
50. Kelly TK, De Carvalho DD, Jones PA (2010) Epigenetic modifications as therapeutic targets. Nat Biotechnol:1069–1078. https://doi.org/10.1038/nbt.1678
51. Kettunen E et al (2017) Asbestos-associated genome-wide DNA methylation changes in lung cancer. Int J Cancer 141(10):2014–2029. https://doi.org/10.1002/ijc.30897
52. Ladd-Acosta C (2015) Epigenetic signatures as biomarkers of exposure. Curr Environ Health Rep 2(2):117–125. https://doi.org/10.1007/s40572-015-0051-2

53. Li JJ et al (2010) Autophagy and oxidative stress associated with gold nanoparticles. Biomaterials 31(23):5996–6003. https://doi.org/10.1016/j.biomaterials.2010.04.014
54. Liou S-HH et al (2017) Global DNA methylation and oxidative stress biomarkers in workers exposed to metal oxide nanoparticles. J Hazard Mater 331:329–335. https://doi.org/10.1016/j.jhazmat.2017.02.042
55. Lu X, Miousse IR, Pirela SV, Moore JK et al (2016a) In vivo epigenetic effects induced by engineered nanomaterials: a case study of copper oxide and laser printer-emitted engineered nanoparticles. Nanotoxicology 10(5):629–639. https://doi.org/10.3109/17435390.2015.1108473
56. Lu X, Miousse IR, Pirela SV, Melnyk S et al (2016b) Short-term exposure to engineered nanomaterials affects cellular epigenome. Nanotoxicology 10(2):140–150. https://doi.org/10.3109/17435390.2015.1025115
57. Ma Y et al (2017) Titanium dioxide nanoparticles induce size-dependent cytotoxicity and genomic DNA hypomethylation in human respiratory cells. RSC Adv 7(38):23560–23572. https://doi.org/10.1039/C6RA28272E
58. Ma Y et al (2019) Different effects of titanium dioxide nanoparticles instillation in young and adult mice on DNA methylation related with lung inflammation and fibrosis. Ecotoxicol Environ Saf 176:1–10. https://doi.org/10.1016/j.ecoenv.2019.03.055
59. Magdolenova Z et al (2014) Mechanisms of genotoxicity. A review of *in vitro* and *in vivo* studies with engineered nanoparticles. Nanotoxicology 8(3):233–278. https://doi.org/10.3109/17435390.2013.773464
60. Manke A, Wang L, Rojanasakul Y (2013) Mechanisms of nanoparticle-induced oxidative stress and toxicity. Biomed Res Int. https://doi.org/10.1155/2013/942916
61. Menezo YJR et al (2016) Oxidative stress and alterations in DNA methylation: two sides of the same coin in reproduction. Reprod Biomed Online:668–683. https://doi.org/10.1016/j.rbmo.2016.09.006
62. Mitchell LA et al (2009) Mechanisms for how inhaled multiwalled carbon nanotubes suppress systemic immune function in mice. Nat Nanotechnol 4(7):451–456. https://doi.org/10.1038/nnano.2009.151
63. Murugadoss S et al (2020) Agglomeration of titanium dioxide nanoparticles increases toxicological responses in vitro and in vivo. Part Fibre Toxicol 17(1):10. https://doi.org/10.1186/s12989-020-00341-7
64. Mytych J et al (2017) Prolonged effects of silver nanoparticles on p53/p21 pathway-mediated proliferation, DNA damage response, and methylation parameters in HT22 hippocampal neuronal cells. Mol Neurobiol 54(2):1285–1300. https://doi.org/10.1007/s12035-016-9688-6
65. Ng CT et al (2011) The induction of epigenetic regulation of PROS1 gene in lung fibroblasts by gold nanoparticles and implications for potential lung injury. Biomaterials 32(30):7609–7615. https://doi.org/10.1016/j.biomaterials.2011.06.038
66. Ngobili TA, Daniele MA (2016) Nanoparticles and direct immunosuppression. Exp Biol Med:1064–1073. https://doi.org/10.1177/1535370216650053
67. Ognik K et al (2019) The effect of copper nanoparticles and copper (II) salt on redox reactions and epigenetic changes in a rat model. J Anim Physiol Anim Nutr 103(2):675–686. https://doi.org/10.1111/jpn.13025
68. Öner D et al (2017) Epigenetic effects of carbon nanotubes in human monocytic cells. Mutagenesis 32(1):181–191. https://doi.org/10.1093/mutage/gew053
69. Öner D, Ghosh M, Bové H et al (2018a) Differences in MWCNT- and SWCNT-induced DNA methylation alterations in association with the nuclear deposition. Part Fibre Toxicol 15(1):11. https://doi.org/10.1186/s12989-018-0244-6
70. Öner D, Ghosh M, Moisse M et al (2018b) Global and gene-specific DNA methylation effects of different asbestos fibres on human bronchial epithelial cells. Environ Int 115:301–311. https://doi.org/10.1016/j.envint.2018.03.031
71. Öner D et al (2020) Induction and recovery of CpG site specific methylation changes in human bronchial cells after long-term exposure to carbon nanotubes and asbestos. Environ Int 137:105530. https://doi.org/10.1016/j.envint.2020.105530
72. Ou L et al (2017) The mechanisms of graphene-based materials-induced programmed cell death: a review of apoptosis, autophagy, and programmed necrosis. Int J Nanomedicine:6633–6646. https://doi.org/10.2147/IJN.S140526
73. Oxidative stress alters global histone modification and DNA methylation (2015) Free Radic Biol Med 82:22–28. https://doi.org/10.1016/J.FREERADBIOMED.2015.01.028
74. Pakrashi S et al (2014) In vivo genotoxicity assessment of titanium dioxide nanoparticles by Allium cepa root tip assay at high exposure concentrations. PLoS One 9(2):e87789. https://doi.org/10.1371/journal.pone.0087789
75. Park EJ, Park K (2009) Oxidative stress and pro-inflammatory responses induced by silica nanoparticles in vivo and in vitro. Toxicol Lett 184(1):18–25. https://doi.org/10.1016/j.toxlet.2008.10.012
76. Park MVDZ et al (2011) The effect of particle size on the cytotoxicity, inflammation, developmental toxicity and genotoxicity of silver nanoparticles. Biomaterials 32(36):9810–9817. https://doi.org/10.1016/j.biomaterials.2011.08.085
77. Patil NA, Gade WN, Deobagkar DD (2016) Epigenetic modulation upon exposure of lung fibroblasts to TiO2 and ZnO nanoparticles: alterations

in DNA methylation. Int J Nanomedicine 11:4509–4519. https://doi.org/10.2147/IJN.S110390
78. Patil YM, Rajpathak SN, Deobagkar DD (2019) Characterization and DNA methylation modulatory activity of gold nanoparticles synthesized by Pseudoalteromonas strain. J Biosci 44(1):1–13. https://doi.org/10.1007/s12038-018-9842-6
79. Patlolla A, Patlolla B, Tchounwou P (2010) Evaluation of cell viability, DNA damage, and cell death in normal human dermal fibroblast cells induced by functionalized multiwalled carbon nanotube. Mol Cell Biochem 338(1–2):225–232. https://doi.org/10.1007/s11010-009-0356-2
80. Pogribna M et al (2020) Effect of titanium dioxide nanoparticles on DNA methylation in multiple human cell lines. Nanotoxicology 14(4):534–553. https://doi.org/10.1080/17435390.2020.1723730
81. Poland CA et al (2008) Carbon nanotubes introduced into the abdominal cavity of mice show asbestos-like pathogenicity in a pilot study. Nat Nanotechnol 3(7):423–428. https://doi.org/10.1038/nnano.2008.111
82. Ray Chaudhuri A, Nussenzweig A (2017) The multifaceted roles of PARP1 in DNA repair and chromatin remodelling. Nat Rev Mol Cell Biol:610–621. https://doi.org/10.1038/nrm.2017.53
83. Rossner P et al (2020) Gene expression and epigenetic changes in mice following inhalation of copper(II) oxide nanoparticles. Nano 10(3):550. https://doi.org/10.3390/nano10030550
84. Roy Choudhury S et al (2017) ZnO nanoparticles induced reactive oxygen species promotes multimodal cyto- and epigenetic toxicity. Toxicol Sci 156(1):kfw252. https://doi.org/10.1093/toxsci/kfw252
85. Saarimäki LA et al (2020) Toxicogenomics analysis of dynamic dose-response in macrophages highlights molecular alterations relevant for multi-walled carbon nanotube-induced lung fibrosis. NanoImpact 20:100274. https://doi.org/10.1016/j.impact.2020.100274
86. Seidel C et al (2017) Epigenetic changes in the early stage of silica-induced cell transformation. Nanotoxicology 11(7):923–935. https://doi.org/10.1080/17435390.2017.1382599
87. Senut M-C et al (2016) Size-dependent toxicity of gold nanoparticles on human embryonic stem cells and their neural derivatives. Small 12(5):631–646. https://doi.org/10.1002/smll.201502346
88. Shukla RK et al (2011) ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. Toxicol In Vitro 25(1):231–241. https://doi.org/10.1016/j.tiv.2010.11.008
89. Shvedova AA et al (2016) Integrated analysis of dysregulated ncRNA and mRNA expression profiles in humans exposed to carbon nanotubes. PLoS One 11(3):e0150628. https://doi.org/10.1371/journal.pone.0150628
90. Sierra MI et al (2017) DNA methylation changes in human lung epithelia cells exposed to multi-walled carbon nanotubes. Nanotoxicology 11(7):857–870. https://doi.org/10.1080/17435390.2017.1371350
91. Singh N et al (2017) Exposure to engineered nanomaterials: impact on DNA repair pathways. Int J Mol Sci. https://doi.org/10.3390/ijms18071515
92. Smith MT et al (2016) Key characteristics of carcinogens as a basis for organizing data on mechanisms of carcinogenesis
93. Stoccoro A et al (2017) Multiple endpoints to evaluate pristine and remediated titanium dioxide nanoparticles genotoxicity in lung epithelial A549 cells. Toxicol Lett 276:48–61. https://doi.org/10.1016/j.toxlet.2017.05.016
94. Tabish AM et al (2017) Changes in DNA methylation in mouse lungs after a single intra-tracheal Administration of Nanomaterials. PLoS One 12(1):e0169886. https://doi.org/10.1371/journal.pone.0169886
95. Unfried K et al (2008) Carbon nanoparticle-induced lung epithelial cell proliferation is mediated by receptor-dependent Akt activation. Am J Phys Lung Cell Mol Phys 294(2):L358–L367. https://doi.org/10.1152/ajplung.00323.2007
96. Vandebriel RJ, De Jong WH (2012) A review of mammalian toxicity of ZnO nanoparticles. Nanotechnol Sci Appl:61–71. https://doi.org/10.2147/NSA.S23932
97. Verleysen E et al (2020) Physicochemical characterization of the pristine E171 food additive by standardized and validated methods. Nano 10(3):592. https://doi.org/10.3390/nano10030592
98. Vlaanderen J et al (2017) A cross-sectional study of changes in markers of immunological effects and lung health due to exposure to multi-walled carbon nanotubes. Nanotoxicology 11(3):395–404. https://doi.org/10.1080/17435390.2017.1308031
99. Wei JW et al (2017) Non-coding RNAs as regulators in epigenetics (review). Oncol Rep 37(1):3–9. https://doi.org/10.3892/or.2016.5236
100. Williams K, Christensen J, Helin K (2012) DNA methylation: TET proteins – guardians of CpG islands? EMBO Rep 13(1):28–35. https://doi.org/10.1038/embor.2011.233
101. Yu KN et al (2013) Zinc oxide nanoparticle induced autophagic cell death and mitochondrial damage via reactive oxygen species generation. Toxicol In Vitro 27(4):1187–1195. https://doi.org/10.1016/j.tiv.2013.02.010
102. Zhao Y, Sun H, Wang H (2016) Long noncoding RNAs in DNA methylation: new players stepping into the old game. Cell Biosci:45. https://doi.org/10.1186/s13578-016-0109-3
103. Zhao X, Toyooka T, Ibuki Y (2017) Silver nanoparticle-induced phosphorylation of histone H3 at serine 10 is due to dynamic changes in actin filaments and the activation of Aurora kinases.

Toxicol Lett 276:39–47. https://doi.org/10.1016/j.toxlet.2017.05.009

104. Ziech D et al (2011) Reactive oxygen species (ROS) – induced genetic and epigenetic alterations in human carcinogenesis. Mutat Res:167–173. https://doi.org/10.1016/j.mrfmmm.2011.02.015

105. Zou Y et al (2016) DNA Hypermethylation of CREB3L1 and Bcl-2 associated with the mitochondrial-mediated apoptosis via PI3K/Akt pathway in human BEAS-2B cells exposure to silica nanoparticles. PLoS One 11(6):e0158475. https://doi.org/10.1371/journal.pone.0158475