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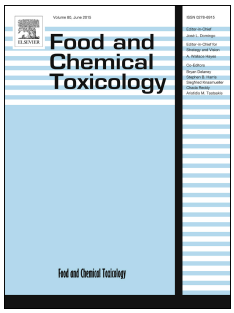

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Epigenetic modulations in nanoparticle-mediated toxicity

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Introduction

Nanoparticles (NPs) are defined as "*particles with sizes between 1 and 100 nm that show properties, which are not found in bulk samples of the same material*" [1]. They can be broadly categorised as airborne ultrafine particles (UFP) and engineered nanoparticles (ENPs). UFPs consist of airborne particulate matter such as dust, soot and smoke that are found in the atmosphere, whereas ENPs are artificial, synthesized NPs. The unique physicochemical properties of ENPs (referred to as NPs from hereon) have made NPs to be utilized in various fields, including healthcare, manufacturing and even food industry [2-7]. For example, NPs are being employed as a useful tool to deliver drugs to disease sites. Nanocapsules coated with chemically modified polyethylene glycol (PEG) chitosan can be used as a nano-sized drug carrier for oral peptide delivery. These nanocapsules exhibited an increased stability in gastrointestinal fluid, thus enhancing the absorption rate of the peptides [8]. NPs are also useful as non-invasive bioimaging tools. Quantum dots (QD), spherical and fluorescent nanocrystals, can be conjugated to proteins to serve as molecular cancer biomarkers and as *in vivo* tumour detection tools in living subjects [9, 10]. However, our constant exposure to NPs has raised concerns of the potential health hazards that they pose. To evaluate the potential risks associated with NPs, a field that specializes in the study of NP toxicity, known as nanotoxicity, has emerged.

Nanotoxicity involves the understanding of adverse biological effects of NPs at cell, tissue, organ and organism levels. Their unique properties such as small sizes and large surface area to volume (SA/V) ratios can promote their reactivity but induce toxicity as well [11]. Over the decades, extensive studies have been conducted to assess the toxic effects of NPs using both *in vitro* and *in vivo* model systems. One of the primary causes of nanotoxicity is known to be the excessive reactive oxygen species (ROS) production through the NPs' interaction with biological molecules such as proteins, lipids and DNA, resulting in oxidative stress [12], lipid peroxidation [13] and inflammation [14]. It has been reported that oxidative stress is observed in human A549 lung cancer cells upon

exposure to cerium oxide nanoparticles (CeO_2 NPs), along with lipid peroxidation and cell membrane damage [15]. In another *in vivo* study using asthmatic model of Balb/c mice, exposure to copper oxide nanoparticles (CuO NPs) was shown to lead an increase in inflammatory cell counts and pro-inflammatory cytokine levels, along with the phosphorylation (activation) of mitogen-activated protein kinase (MAPK), a downstream target activated by inflammatory cytokines. Notably, the development of asthma in these mice was aggravated, highlighting the potential toxic effects of NPs in human health [16]. Furthermore, the testes of the fruitfly *Drosophila melanogaster* exposed to silver nanoparticles (AgNPs) showed an increased ROS production, which led to a loss of germline stem cells (GSCs) by promoting precocious GSC differentiation [17]. NPs are also known to infiltrate into cell nuclei, causing genotoxicity by directly damaging DNA [18]. In human mesenchymal stem cells, AgNPs were detected in the nuclei and damaged DNA in a time-dependent manner [19].

More recently, it was reported that NPs inevitably interact with biological materials upon entering the human bodies [20]. Molecules such as serum/plasma cellular proteins in the bodies adsorb onto the surfaces of NPs and form a layer of coating around them, forming protein coronas. NPs then cause the conformational changes of the adsorbed proteins which allow them to interact with cell membrane proteins, facilitating the uptake of NPs into the cells. For example, a study investigating the interaction of gold nanoparticles (AuNPs) with serum albumin in a cell-free system revealed that AuNPs induced conformational changes of serum albumin, led to an increased AuNP uptake [21]. Once protein coronas enter the cells, they can induce cytotoxicity by facilitating the degradation of intracellular proteins. Nanoparticles are also known to cause toxic effects by promoting protein citrullination, a process that converts arginine residues of proteins into citrullines which results in a net loss of a peptide's positive charge and thus, leads to a loss of functionality of the proteins [22]. Silicon dioxide nanoparticles (SiO_2 NPs), carbon black and single-walled carbon nanotubes (SWCNTs) were also found to cause protein citrullination in human A549 and phagocytic THP-1 cells [23].

Interestingly, there is an increasing body of evidences that suggest the heritability nature of NP-mediated toxicity [24-28]. Since the offspring carries the same genetic makeup as their parents, the most likely explanation is that NP exposure has affected the epigenetic prints in these offspring. Here, we discussed about the NP-mediated epigenetic modifications in the aspects of DNA methylation, histone modification and non-coding RNA expression.

Epigenetics

Epigenetics is "*the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence*" [29]. Decades of studies have revealed that DNA expressions can be regulated without altering the underlying sequences via several mechanisms, namely DNA methylation, histone modifications and non-coding RNAs. These findings challenged the role of DNA sequence variants as the only source of heritable phenotypes. Epigenetic modifications are heritable and remain persistent after initial stimulus exposure through the retention of epigenetic memory [30, 31]. To ensure successful and continuous reproduction, the robustness and fidelity of epigenetic modification are essential. However, they are constantly being subjected to environmental stress which leads to the manifestation of adverse phenotypes. As nanoparticles become more prevalent in our lives, they act as a form of environmental perturbation to the homeostasis of the epigenome, suggesting the potential long-term adverse effects of NPs on human health.

Nanoparticle-induced alterations in DNA methylation

Studies have shown that NP exposure can induce changes in DNA methylation patterns. DNA methylation is the addition of a methyl group to the 5^{th} carbon of a cytosine residue to form 5' methylated cytosine (5mc). NPs can alter DNA methylation patterns by modulating the expression and activity of DNA methyltransferases (DNMTs), a family of enzymes that catalyse DNA methylation.

NP-induced hypermethylation at gene promoters blocks gene expression, while promoter demethylation facilitates gene expression [32].

In vertebrates, DNA is predominantly methylated at the dinucleotide CpG sites in which a cytosine base is separated from a guanine base by phosphate linkage. The strategic positioning of CpG islands (unmethylated regions of DNA with high densities of CpG) at the 5' ends of most genes allows DNA methylation to act as a regulator of transcription initiation [33, 34]. Methylated DNA sequences prevent the binding of transcription machinery to target genes, thereby inhibiting their expressions. Likewise, repressors such as methyl-CpG-binding domain (MBD) family, which recognize methylated-CpG can prevent the transcription of target genes as well [35]. Therefore, NP-mediated changes in DNA methylation patterns at these CpG islands can significantly alter gene expressions. In human bronchial epithelial BEAS-2B cells exposed to silica NPs, approximately 5% of the CpG loci showed differential methylation levels. From these 5%, *CREB3L1 (CAMP Responsive Element Binding Protein 3 Like 1*) and *Bcl-2* (*B-cell lymphoma 2*) genes were identified to be significantly hypermethylated, along with the decreased expressions of the respective genes at protein levels [36]. Interestingly, Choudhury *et al.* investigated locus-specific methylation patterns in ZnO NP-exposed cells on several genomic repeat sequences in human embryonic kidney 293 (HEK-293) cells and found a significant hypomethylation at *LINE-1 (long interspersed nucleotide element 1)* [37]. This finding aligns well with another study, which reported decreased DNA methylation levels in leukocytes *LINE-1* sequences of elderly individuals upon a short term exposure to airborne UFPs from traffic emissions [38].

Notably, DNA methylation changes are not always the same; they differ according to the type of NPs and cells used. Exposure to printer-emitted engineered nanoparticles (PEPs), mild steel welding fumes (MS-WF), CuO NPs or titanium dioxide nanoparticles (TiO_2 NPs) resulted in both hypo- and hypermethylation, depending on the cell type used and the genomic repeat sequences investigated

[39]. Similarly, exposure of Balb/c mice to AuNPs, SWCNTs or multi-walled carbon nanotubes (MWCNTs) exhibited differential methylation changes based on the shapes and sizes of NPs. For instance, differences in the shapes and sizes of CNTs and AuNPs led to various levels of DNA methylation at *Atm (ataxia telangiectasia mutated)* and *trp53 (tumour protein p53)*, respectively. Researchers also reported differential methylation patterns by NPs in various gene loci. 60 nm AuNPs were shown to induce hypermethylation in the promoters of *Atm*, *Cdk (cyclin-dependent kinase)* and *Gsr (glutathione reductase)*, but hypomethylation in the promoter of *Gpx (glutathione peroxidase)* [40].

To estimate global DNA methylation levels, the methylation levels of repeated DNA sequences can be used as a surrogate marker [41]. While this may not accurately reflect the alterations in specific gene loci, it is still a useful benchmark to gauge overall nanoparticle-induced methylation changes. A recent study investigated the effect of occupational exposure to metal oxide NPs on global DNA methylation. These NP-handling workers from Taiwan were constantly exposed to TiO_2, SiO_2 or indium tin oxide (ITO) NPs. Interestingly, those exposed to ITO NPs showed lower global DNA methylation levels along with elevated oxidative stress, whereas those exposed to SiO_2 or TiO_2 NPs did not exhibit any significant global hypomethylation but induced oxidative damages [42]. However, an independent study showed that SiO_2 NPs induced global DNA hypomethylation in human HaCaT cells, along with decreased mRNA levels of *DNMT1* (*DNA Methyltransferase 1*) and *DNMT3a* (*DNA Methyltransferase 3a)* [43]. One possible explanation for this discrepancy is that the exposure level or duration in the Taiwanese workers may not be high enough to induce epigenetic alterations.

NP exposure can also affect the expression or activity of DNMTs, which are well known for their roles in causing changes in the pattern of DNA methylation. While DNMT1 is responsible for the replication of DNA methylation pattern during DNA replication, DNMT3a and DNMT3b act to facilitate the establishment of new methylation pattern in unmodified DNA [44]. An augmentation in

DNA methylation upon NP exposure usually corresponds with an increase in DNMT levels, and this effect is reversible by knocking down *DNMT* in affected cells [45, 46]. Similarly, exposure of human MRC5 lung fibroblasts to TiO_2 or ZnO NPs induced a dose-dependent hypomethylation of the cell's genomic DNA, with a significant downregulation of *DNMT* transcripts [47]. However, a recent study reported contradictory observation, where a decrease in DNMT expression induced by carbon-based NPs led to a hypermethylation of global genome [48]. Nevertheless, DNMTs are clearly responsible for DNA methylation, but the exact molecular mechanism underlying DNA demethylation by DNMTs remains uncertain. The role of TET1 (Ten-Eleven Translocation 1), which functions to convert 5mc to 5' hydroxymethylcytosine (5hmc), as a DNA demethylase via the base-excision repair pathway was reported in 2011 using adult mouse brain as a model system [49]. Aligned with this finding, a more recent study yielded comparable results where the activity of TET enzymes was increased in ZnO NP-exposed HEK293 cells with a concurrent decrease in global DNA methylation levels, signifying the important role of TET enzymes in DNA methylation changes [37].

Nanoparticle-induced histone modification

As we gain more insights into our understanding of NP-mediated DNA methylation changes, more researchers also begin to investigate the possible effects of NPs on histone modifications. Histones belong to a class of nuclear proteins that have globular octameric structures, comprising of two copies of H2A, H2B, H3 and H4 histones each. Histones aid in tighter packaging of DNAs in the nucleus by allowing DNA to wrap around them to form a basic unit called nucleosome [50]. The tightness of DNA packaging relies on several types of modifications on the amino (N)-terminal tails of histones, which protrude from the nucleosomes, thus influencing transcription of nearby genes. These modifications comprise of acetylation, methylation, phosphorylation, sumoylation and ubiquitylation. However, only histone acetylation, methylation and phosphorylation have so far been reported in NP-related studies [51].

One of the most well studied mechanisms of NP-mediated histone modification is histone acetylation, the process of adding an acetyl group to lysine (K) residue on histones. It is mediated by a family of enzymes called histone acetyltransferases (HATs) [52]. Unlike DNA methylation, the removal of acetyl groups by another family of enzymes known as histone deacetylases (HDACs) is well characterized [53]. Histone acetylation is widely associated with increased transcriptional activity, whereas histone deacetylation causes chromatin condensation, making it inaccessible to transcription factors and the genes are thus silenced [54]. In 2007, global hypoacetylation of H3 in human MCF7 breast cancer cells upon a short term exposure to QDs was first reported. Consistently, authors observed chromatin condensation in the nuclei of the cells which is attributed to the removal of acetyl groups from H3 [55]. Similarly, exposure of human MRC5 fetal fibroblasts to AuNPs was reported to cause chromatin condensation in the cells [56]. Interestingly, QD-induced hypoacetylation was shown to be reversible through the action of trichostatin A, a HDAC inhibitor, suggesting the role of HDACs in NP-induced histone modification [55]. It is worth to note that the anticancer drug gefitinib, loaded in poly lactic-co-glycolic acid (PLGA) nanoparticles was found to hyperacetylate histone H3 in human lung carcinoma cells via the activation of histone acetyltransferases, which in turn promoted cell death [57]. This finding represents a crucial stepping stone in healthcare advancements as it shed light on the potential use of nanoparticles to combat diseases such as cancer via epigenetic modifications. The lysine residue on histones are also subjected to methylation and/or demethylation reactions mediated by NPs which in turn affects gene transcriptions. This process is catalysed by methyltransferases and/or demethyltransferases [52]. In mouse erythroleukemia cells, AgNP exposure was shown to induce a significant decrease in H3K4 and H3K79 methylation levels [58], while AuNPs caused a downregulation of H3K27 trimethylation in human small airway epithelial cells [59].

While most of the studies investigated only one type of histone modification, NPs may induce multiple histone modifications simultaneously. HaCaT cells treated with ZnO NPs showed condensed

nuclei and decreased acetylation of H4 at K5 residues. Concurrently, an upregulation of histone methylation at H3K9, along with the expression of histone methyltransferases was also detected [60]. Similarly, exposure of arsenic trioxide NPs to the human prostate LNCaP and PC-3 cancer cells resulted in an increase in acetylation at H3K14 and phosphorylation at H3 serine 10 residue, but a decrease in H3K9 methylation [61]. Another study using healthy, male workers who are occupationally exposed to particulate matter containing metal components (consisting of aluminium, manganese, nickel, zinc, arsenic, lead, iron) also reported an increase in both H3K9 acetylation and H3K4 methylation levels [62]. These studies highlight the crosstalk between distinct types of histone modification.

Nanoparticle-induced alterations in miRNA Expression

With the advent of high throughput technology, gene expression profiling has been utilized on multiple occasions to better understand the effects of NPs on the dysregulation of microRNAs (miRNAs). A miRNA is a short non-coding RNA that does not translate into proteins but exerts its inhibitory effects on gene transcription based on complimentary binding of miRNAs to target mRNAs at the 'seed' region, which consists of nucleotides 2-8 at 5' end of miRNAs. Complementary binding at this region leads to a miRNA-mediated mRNA decay, while partial complementarity leads to an inhibition of translation elongation [63, 64].

Several studies have reported the effects of NPs on miRNA regulation. In A549 cells, TiO_2 NP exposure led to a persistent downregulation of miRNA-21 and miRNA-30a, both of which function in autophagy regulation [65]. In human dermal fibroblasts, AgNP exposure was found to alter the expression of 6 distinct miRNAs, which function to regulate cell survival by inducing the degradation and/or translational inhibition of mRNAs and/or proteins related to apoptosis, suggesting the cytotoxic effects of AgNPs by altering miRNA expression patterns [66]. However, the same group of researchers later revealed that while AuNPs can induce differential miRNA expressions in human

dermal fibroblasts, they do not induce cytotoxicity as observed in AgNPs [67], suggesting that different types of NPs can affect different sets of miRNAs. Importantly, AuNP administration in pregnant Swiss mice exhibited a significant upregulation of Let-7a and miR-183 in both the liver and peripheral blood of mice foetuses, suggesting the heritability of epigenetic toxicity along with their health consequences [68].

Particulate matter (PM) in our atmosphere has also been shown to cause an alteration in miRNA expression levels. In a recent pilot study, a long-term exposure of ambient PM2.5 led to elevated extracellular vesicles-encapsulated miRNA levels (containing miR-126-3p, miR-19b-3p, miR-93-5p, miR-223-3p and miR-142-3p) in the serum samples of participants selected from the US Department of Veterans Affairs Normative Aging Study [69]. In an independent study, a short-term exposure of school students to UFPs showed an upregulation of saliva extracellular miR-222 [70]. While the health consequences of these miRNA upregulation remain to be elucidated, these miRNAs have been identified as biomarkers in cardiovascular diseases, thus suggesting the connection between exposure of pollutants and the development of cardiovascular diseases [71, 72]. Furthermore, other studies have shown that cigarette smoke and carbon black can increase the expression of miR-135b, which has been associated with inflammation, cancer and other diseases, in exposed mice [73-75]. Similarly, mice exposed to TiO_2 NPs for 11 consecutive days exhibited an upregulation of 16 distinct types of miRNAs, with miR-135b recorded the highest fold of increase by 60 times [76]. Other studies have also reported a correlation between NP-mediated miRNA dysregulation and tumorigenesis. Diesel exhaust particles (DEP), one of the largest sources of emitted PM in our atmosphere, was shown to induce miRNA dysregulation in human airway epithelial cells which in turn led to lung tumour progression [77]. Similarly, in human bronchial epithelial cells, DEP was shown to induce an upregulation of miR-21 expression and an activation of the PTEN/PI3K/AKT pathway, an important carcinogenic pathway [78]. DEP and PM were also reported to contribute to the adaptive immune disorders through thymic stromal lymphopoietin

upregulation induced by hsa-miR-375 [79]. Taken altogether, these studies suggest that dysregulation of miRNA expression caused by UFPs and/or NPs may contribute to the pathogenesis of various human diseases.

High throughput technology has enabled the investigation of alterations in miRNA levels. However, these findings are superfluous if the downstream effects and consequences remain unknown. Therefore, researchers have combined gene expression profiling with pathway analysis to better understand the canonical signalling pathways affected by NP-mediated miRNA dysregulation. For instance, integrated analysis of miRNA and mRNA expression profiling in Jurkat T cells after AgNP exposure revealed that miR-219-5p regulates the expressions of MT1F (metallothionein 1F) and TRIB3 (tribbles homolog 3), and that these interactions are involved in various cellular processes such as cell cycle and cell death [80]. The human lung carcinoma GLC-83 cells and *C. elegans* exposed to graphene oxide NPs and MWCNTs, respectively, also showed an alteration in miRNA expression. Importantly, the potential target genes that may be affected by these miRNAs were identified to be involved in a wide range of biological processes such as cell cycle, cell metabolism, development and apoptosis [81, 82]. Similarly, in NIH/3T3 cells, cadmium-telluride quantum dots (CdTe QDs) were found to induce cytotoxicity via the dysregulation of miRNAs such as miR-222 and let-7a [83]. Another study also showed that NIH/3T3 cells exposed to several NPs separately, namely iron oxide NPs, CdTe QDs and MWCNTs, exhibit an altered expression of miRNAs, which are known to be involved in nanomaterial-mediated cytotoxicity [84]. These findings further emphasize the importance of parallel analysis for a better understanding of NP-mediated biological effects.

Conclusion and Future Perspectives

The rise in the use of NPs has led to in-depth studies on their toxicity. In recent years, the potential adverse effects of NPs on epigenetics are gaining attention. Figure 1 summarizes cytotoxicity and epigenetic modifications induced by various NPs, based on the information available

in literatures. However, these findings have so far only represented the tip of the iceberg (Table 1) with so much to explore still.

Several studies have reported alterations in DNA methylation and histone acetylation by NP exposure. However, we are still lacking much information on other types of histone modifications such as methylation, phosphorylation and sumoylation. Similarly, miRNA has been focused in nanotoxicity studies, but there are several different types of other ncRNAs that might have been affected as well. Moreover, most studies have focused on only one type of epigenetic modification by one type of NPs, but elucidating the interplay between different epigenetic modifications by the same NPs might provide more comprehensive insights into our understanding of nanotoxicity. The specific role of NPs' physicochemical properties such as sizes and surface charges in modulating the epigenome also remains largely unexplored.

Another gap that remains to be addressed would be the trans-generational effects of NP exposure on epigenetics. Longitudinal studies are necessary to understand the repercussions of NP-mediated epigenetic modifications and to determine if these effects remain persistent over many generations. In this area, model organisms such as *Drosophila melanogaster* can serve as a useful *in vivo* model for understanding the fundamental epigenetic mechanisms in NP-mediated toxicity and investigating the trans-generational effects of NP exposure. The short life cycle of *Drosophila* with distinct developmental stages allows trans-generational studies to be performed in a relatively brief period of time [85].

Most importantly, dysregulation of epigenetics has been shown to develop into pathological diseases such as cancer and neurodegenerative diseases. Thus, it is not a farfetched extrapolation that NP-mediated epigenetic modulations can similarly lead to pathogenesis to a certain extent. For instance, AgNP exposure led to an increase in miR-1275 and miR-132-3p levels in human embryonic

stem cell (hESC)-derived neural stem/progenitor cells, along with a significant downregulation in their target genes (*ADAMTS9* and *SHANK2*), which encode products involved in axonal guidance signalling and whose aberrant expressions are closely associated with neural damage in the brain and neurodegenerative diseases [86]. Global DNA hypomethylation, one of the hallmarks of cancer, can be passed down to future progenies once acquired [37]. Therefore, addressing the functional significance and identifying epigenetic signatures induced by distinct types of NP (as biomarkers) are important challenges for the future.

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Disclosure

The authors declare no conflicts of interest in this work.

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Table 1. Summary of epigenetic modifications mediated by nanoparticles.

Nanoparticles	Sizes	Biological Systems	Epigenetics Modifications	Ref.
Arsenic Trioxide	86 nm	Prostate cancer LNCaP and PC-3 cells	Dose-dependent decrease in mono, di and tri-methylation of H3K9 Dose-dependent increase in H3K14 acetylation Dose-dependent increase in H3S10 phosphorylation	[61]
Carbon	1 nm	Human A549 lung cancer cells	Global genomic DNA hypermethylation with reduced *DNMT* expressions	[48]
	14 nm	C57BL/6 female mice	Upregulation of miR-135b expressions in both the dams and non-pregnant females, but miR-146b and miR-21 were only upregulated in non-pregnant females only	[74]
Single Walled Carbon Nanotubes (SWCNT)	1.2 – 1.5 nm	Human A549 lung cancer cells	Global genomic DNA hypermethylation with reduced *DNMT* expressions	[48]
	-	Balb/c mice	Promoter hypermethylation in *Atm* gene	[40]
Multi Walled Carbon Nanotubes (MWCNT)	10 – 170 nm	Human A549 lung cancer cells	Global genomic DNA hypermethylation with reduced *DNMT* expressions	[48]
	10 – 20 nm	*Caenorhabditis elegans*	Dysregulation of many miRNA expressions whose targets are involved in various biological processes	[82]
Copper Oxide (CuO)	58.7 nm	SAEC	Hypermethylation in LINE-1 sequence and *Alu* elements; Increase in *DNMT1* expression	[39]
		THP-1	Hypermethylation in LINE-1 sequence and *Alu* elements	
		RAW264.7 macrophages	Weak hypomethylation in LINE-1 sequence but modest hypermethylation in SINE B1 elements; Reduced expression of *Tet2*	
Gold (AuNP)	60 nm	Balb/c mice	CpG hypermethylation in *Atm, Cdk* and *Gsr* genes; CpG hypomethylation in *Gpx*	[40]
	20 nm	Human fetal lung fibroblasts	Significant upregulation of miR-155, concomitant with downregulation of *PROS1* gene, which can lead to thrombosis in pulmonary vasculature	[56]
	20 nm	Small airway epithelial cells	Decreased H3K27 trimethylation	[59]
Quantum Dots (QD)	-	Human MCF7 breast cancer cells	Chromatin condensation and global hypoacetylation of H3	[55]
	-	NIH/3T3 cells	Dysregulation in expressions of >200 miRNA	[83]

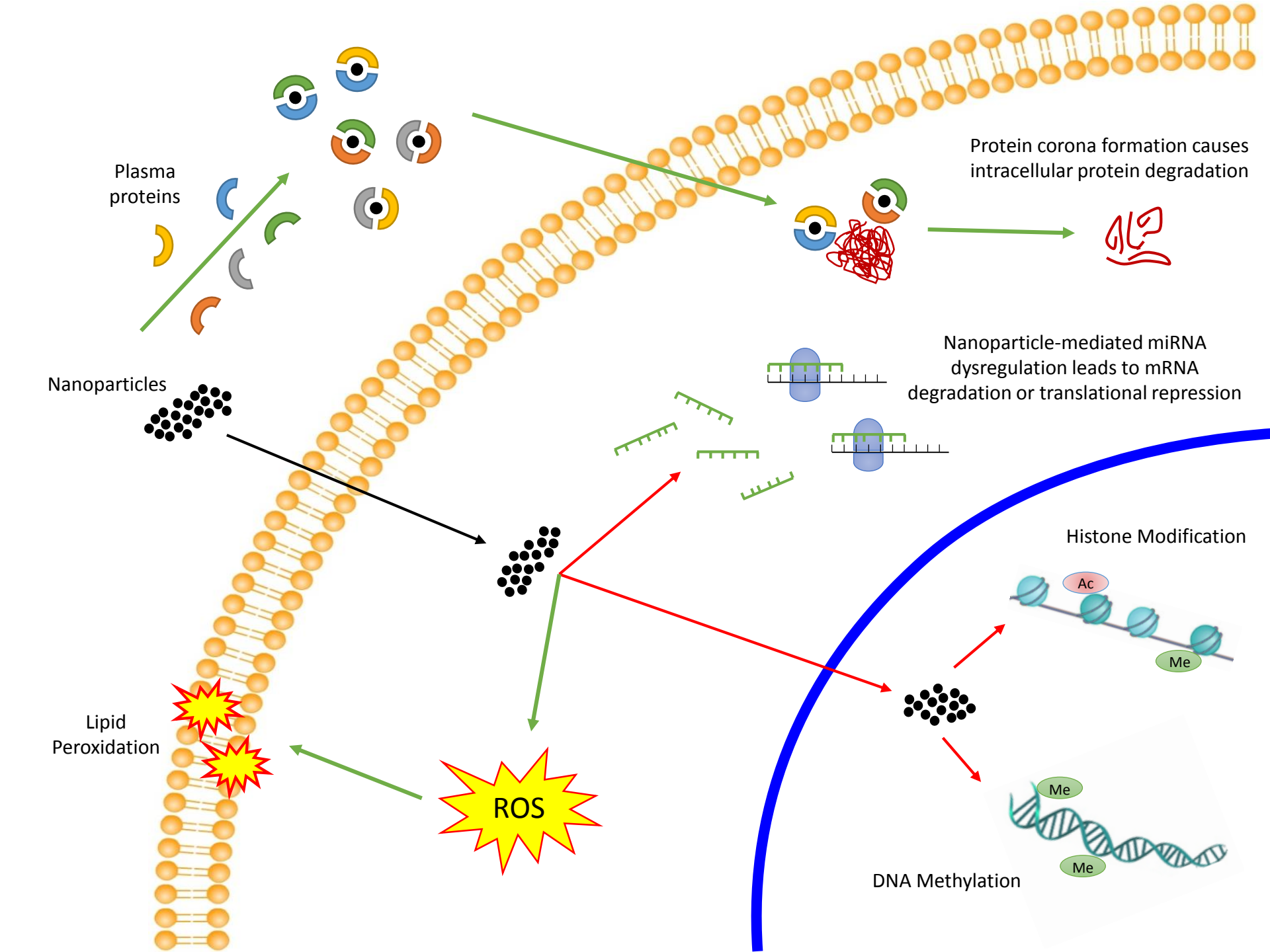
Table 1. Summary of epigenetic modifications mediated by nanoparticles.

Nanoparticles	Sizes	Biological Systems	Epigenetics Modifications	Ref.
Silicon Dioxide (SiO_2)	15 nm	Human keratinocytes HaCaT cells	Global DNA hypomethylation with downregulation of *DNMT1* and *DNMT3a* genes	[43]
Silver (AgNP)	25 nm	Mouse erythroleukemia cells	Decreased global H3 methylation and histone methyltransferases levels	[58]
	< 100 nm	Jurkat T Cells	Negative correlation between miR-219-5p with metallothionein 1F (MT1F) and tribbles homolog 3 (TRIB3) mRNAs	[80]
	< 100 nm	Human MCF 7 breast cancer cells, human keratinocytes HaCaT cells, human A549 lung cancer cells	Increased phosphorylation of H2AX	[87]
	200 nm	Human keratinocytes HaCaT cells	Increased phosphorylation of H3S10	[88]
	8 nm	H9 human embryonic stem cells	Upregulation of miR-1275 and miR-132-3p along with reduced expressions of their target genes (*ADAMTS9* and *SHANK2*)	[86]
Titanium Dioxide (TiO_2)	21 nm	Small airway epithelial cells	Increased transcripts of LINE-1 sequence and *Alu* element; decreased expression of *Tet1-Tet3* genes	[39]
		RAW264.7 macrophages	Increased expression of *Tet2*	
	< 100 nm	Lung fibroblasts MRC5 cells	Hypomethylation of global DNA with reduced DNMT transcript levels	[47]
	20 nm	Female C57BL/6BomTac mice	Upregulation of 16 distinct types of miRNAs, with miR135-b recorded a 60-fold increase	[75]
Zinc Oxide (ZnO)	90 nm	Human embryonic kidney HEK-293 cells	DNA hypomethylation at LINE-1 sequences	[37]
	< 100 nm	Lung fibroblasts MRC5 cells	Hypomethylation of global DNA with reduced DNMT transcript levels	[47]
	< 100 nm	Human keratinocytes HaCaT cells	Nuclei condensation with hypoacetylation at H4K5 but increased methylation at H3K9 and histone methyltransferases levels	[60]

Figure legend

Figure 1. Nanoparticle-induced cytotoxicity and epigenetic modification. Nanoparticles interact with biological molecules, resulting in cytotoxicity and epigenetic modification. Nanoparticle-induced cytotoxicity includes oxidative stress and corona effect, while nanoparticle-mediated epigenetic modification includes alterations in DNA methylation pattern, histone modification and miRNA expression.

Ac: Acetylated; Me: Methylated; ROS: Reactive oxygen species


Plasma proteins
Protein corona formation causes intracellular protein degradation
Nanoparticles
Nanoparticle-mediated miRNA dysregulation leads to mRNA degradation or translational repression
Histone Modification
Ac
Me
Lipid Peroxidation
ROS
Me
Me
DNA Methylation