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# Epigenetic effects of nano-sized materials

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ABSTRACT

The term epigenetics includes several phenomena such as DNA methylation, histone tail modifications, and microRNA mediated mechanisms, which are able to mold the chromatin structure and/or gene expression levels, without altering the primary DNA sequence. Environmental agents can exert epigenetic properties and there is increasing evidence of epigenetic deregulation of gene expression in several human diseases, including cancer, cardiovascular diseases, autism spectrum disorders, autoimmune diseases, and neurodegeneration, among others. Given the widespread use and dispersion in the environment of nano-sized materials, this article summarizes the studies performed so far to evaluate their potential epigenetic properties. Those studies highlight the ability of certain nano-sized compounds to induce an impaired expression of genes involved in DNA methylation reactions leading to global DNA methylation changes, as well as changes of gene specific methylation of tumor suppressor genes, inflammatory genes, and DNA repair genes, all potentially involved in cancer development. Moreover, some nano-sized compounds are able to induce changes in the acetylation and methylation of histone tails, as well as microRNA deregulated expression. We also provided a detailed description of currently available methodologies to evaluate epigenetic modifications. Standard protocols are currently available to evaluate cytotoxic and genotoxic effects of nano-sized materials. By contrast, there are at present no available standard protocols to evaluate the epigenetic potential of any given compound. The currently available methodologies offer different, but often complementary information to characterize potential epigenetic changes induced by exposure to nano-sized compounds. Given the widespread use and dispersion in the environment of nano-sized materials, at present and foreseeable in the near future, and in light of the indication of potential epigenetic properties here reviewed, more attention should be paid to unravel the consequences of such effects in future studies.



## 1. Introduction

A major concern applying to the use and handling of engineered nanomaterials (ENMs) is that their toxicity is likely to be due to physicochemical properties that standard toxicity screening tests are not able to evaluate. Several physicochemical factors have been related to biological responses; *e.g.*, size, surface area, high aspect ratio, charge, solubility, surface chemistry and reactivity. Progresses have been made in identifying priorities or minimal analytical characterization of ENM needed for hazard assessment in biological matrices (Bouwmeester et al., 2011). At present the OECD and the USEPA guidelines for the investigation of high production volume chemicals represent the reference model for assessing toxicity of ENM, although the OECD has published two documents specifically devoted to this subject: the first was published in 2009 ("Guidance Manual for the Testing of Manufactured Nanomaterials" (ENV/JM/MONO(2009)) and the second "Guidance Manual for the Testing of Manufactured Nanomaterials: First revision" was published in June 2010 (ENV/JM/MONO(2009)20/REV). These documents are considered to be living documents and subjected to regular review, revision and improvement as the state of the art advances. Therefore it is important to provide input for future development of methods which should be considered for future investigation, aiming anyway at improving our knowledge about potential effects exerted by ENM.

Recently there is growing interest in the potential that the environment may have not only against the genome (mutations) but also against epigenome (epimutations). The topic of how epigenetic processes can significantly modulate cellular behavior and potentially complex diseases risk, including cancer, especially in response to environmental chemicals, is just such an emerging issue (Preston, 2007).

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Epigenetics investigates heritable changes in gene expression occurring without changes in DNA sequence. Several epigenetic mechanisms, including DNA methylation, histone tails modifications, and non-coding RNA expression, that regulate gene expression through altering chromatin configuration, inhibition of translation, and degradation of RNA, can change genome function under exogenous influence (Cyr and Domann, 2011). It is well established that long-term toxicity of chemicals could be caused by their ability to generate changes in the DNA sequence through the process of mutagenesis. However DNA function and health could be stably altered by exposure to environmental agents without changing the sequence, just by changing the state of DNA methylation. The majority of environmental factors such as nutrition, or toxicants such as endocrine disruptors, do not promote genetic mutations or alterations in DNA sequence. However, these factors do have the capacity to alter the epigenome. Moreover some environmental factors are able to promote a phenotype or disease state not only in the individual exposed but also in subsequent progeny for successive generations (transgenerational inheritance). Epimutations in the germline that become permanently programmed can allow transmission of epigenetic transgenerational phenotypes (Skinner et al., 2010).

This has important implications on the way we assess the safety of chemicals, drugs and food and broadens the scope of definition of toxic agents (Preston, 2007; Szyf, 2011). *In vitro*, animal and human investigations have identified several classes of environmental chemicals able to modify epigenetic marks, including metals (cadmium, arsenic, nickel, chromium, and methylmercury), peroxisome proliferators (trichloroethylene, dichloroacetic acid, and trichloroacetic acid), air pollutants (particulate matter, black carbon, and benzene), and endocrine-disrupting/reproductive toxicants (diethylstilbestrol, bisphenol A, persistent organic pollutants, and dioxin) (Cheng et al., 2012; Christensen and Marsit, 2011; Szyf, 2011). Most studies conducted so far have been centered on DNA methylation, whereas only a few investigations have studied environmental chemicals in relation to histone modifications and non-coding RNA. Our current screening tests however are not properly addressed to detect agents that have epigenetic properties. In view of a broad environmental health research strategy aimed at protecting and improving human health we should advance our understanding of the potential epigenetic role of ENM.

## 2. Epigenetics: concept and mechanisms

Epigenetics, literally meaning "above genetics", comprises heritable modifications that alter gene expression levels without resulting from direct changes in the primary DNA sequence. Epigenetic mechanisms include nucleotide modifications, such as methylation and hydroxymethylation of cytosine, covalent modifications of histone tails, and nucleosome positioning. These mechanisms interact to determine chromatin folding and the relative accessibility of a given genetic locus to activating and suppressing transcription factors. Non-coding RNAs affecting gene transcription are also largely recognized as epigenetic mechanisms (Martín-Subero, 2011). A summary of major epigenetic processes is shown in Fig. 1.

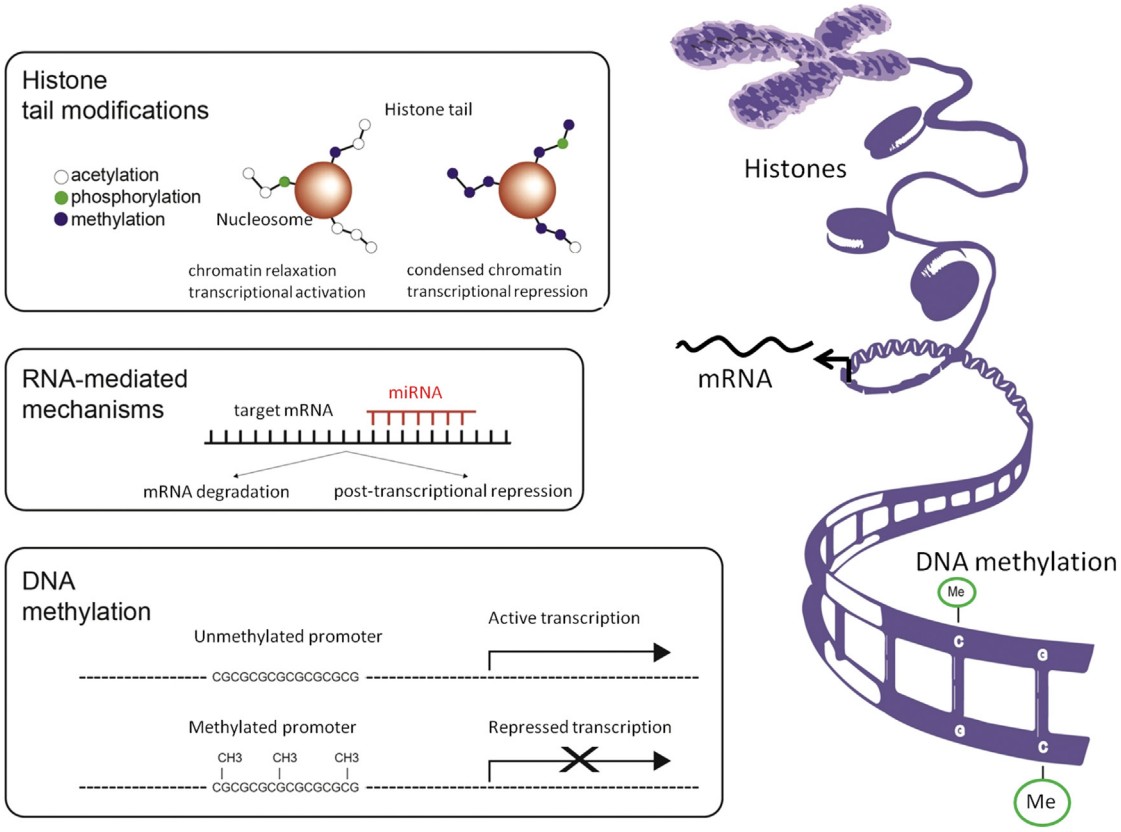


**Fig. 1.** Overview of the main epigenetic mechanisms that can undergo deregulation following exposure to nano-sized materials. Histone tail modifications lead to a more or less relaxed chromatin structure thus allowing or blocking the access of transcription factors to gene promoters. DNA methylation of the promoter regions is generally associated with gene silencing, while demethylated promoters allow the binding of transcription factors and the gene is transcribed into messenger RNA (mRNA). Following post-transcriptional processing the mature mRNA reach the cytoplasm. A microRNA (miRNA) can bind the target mRNA, leading to its degradation or blocking the access to the ribosome and translation into proteins.

### 2.1. Methylation and hydroxymethylation of cytosine

DNA methylation represents one of the most important epigenetic mechanisms for gene regulation. Methylation of cytosines within CpG sequences, and their subsequent interaction with methyl-CpG binding proteins (MBDs), may induce chromatin conformational modifications and inhibit the access of the transcriptional machinery to gene promoter regions, thus altering gene expression levels. Promoter hypermethylation is commonly associated with gene silencing and promoter demethylation with gene expression (Fig. 1) (Martín-Subero, 2011). Moreover, aside from DNA methylation in promoter regions, methylation of intragenic regions (also known as gene body methylation) might have a role in regulating gene expression (Ball et al., 2009). Folate metabolism is a complex pathway required for the production of S-adenosylmethione (SAM), the major intracellular methylating agent, and DNA methylation reactions depend on SAM availability. DNA methyltransferases (DNMTs) catalyze the transfer of a methyl group from SAM to cytosine, thus forming 5-methyl-cytosine. In mammals DNMT1 is primarily involved in the maintenance of DNA methylation patterns during development and cell division, whereas DNMT3a and DNMT3b are the *de novo* methyltransferases and establish DNA methylation patterns during early development. DNMT3a and DNMT3b might also participate in the maintenance of DNA methylation patterns by correcting errors left by DNMT1 (Jones and Liang, 2009). DNMT3L induces *de novo* DNA methylation by recruitment or activation of DNMT3a, while DNMT2 is primarily involved in the methylation of transfer RNA molecules (Goll and Bestor, 2005). In addition to their role in *de novo* DNA methylation, DNMT3a/b have been also involved in dynamic demethylation processes, thus allowing a transient regulation of the transcription of several genes (Métivier et al., 2008).

DNA demethylation processes are still under investigation. Guo et al. (2011a) demonstrated that the 5-methylcytosine hydroxylase enzyme TET (ten–eleven translocation), by converting 5-methylcytosine to 5-hydroxymethylcytosine (5-hmc), promotes DNA demethylation in mammalian cells through a process that requires the DNA base excision repair pathway. Demethylation of 5-hmc is promoted by the AID (activation-induced deaminase)/APOBEC (apolipoprotein B mRNA-editing enzyme complex) family of cytidine deaminases (Guo et al., 2011a). 5-hmc is also referred as the sixth DNA base, is present at high levels in the brain, and its lower affinity to methyl-binding proteins as compared to 5-methylcytosine suggests that it might have a different role in the regulation of gene expression, while it is also implicated in the DNA demethylation process. Indeed, evidence is currently accumulating that 5-hmc may not exclusively be an intermediate of an active demethylation process, but that it functions instead as an important epigenetic marker (Münzel et al., 2011).

### 2.2. Histone tail modifications

The chromatin state represents another important modulator of gene expression profiles. Chromatin can exist in a non-condensate and transcriptionally active state (euchromatin) or in a condensate inactive state (heterochromatin). Conformational changes in histone proteins or modifications of the way in which DNA wraps around the histone octamer in nucleosomes may either alter or facilitate the access of the transcriptional machinery to the promoter region of some genes, leading to gene silencing or activation, respectively. Several post-translational modifications of histone tails are known, including acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, and others. Histone tail acetylation represents one of the most studied modifications and is associated with chromatin relaxation and transcriptional activation, while deacethylation is related to a more condensed chromatin state and transcriptional repression (Fig. 1). Histone acetyltransferases (HATs) catalyze the acetylation of lysine residues in histone tails, whereas histone deacetylation is mediated by histone deacetylases (HDACs) (Berger, 2007). Another frequently studied modification of histone tails is methylation on either lysine or arginine residues. Methylation of histone tails can be associated with either condensation or relaxation of the chromatin structure, since several sites for methylation are present on each tail thus allowing several combinations (Martin and Zhang, 2005). Albeit the exact effects of all potential combinations of posttranslational modification of histone tails (the so called “histone code”) on gene expression are complicated and still being worked out, there is evidence that methylation at H3K4, H3K36 and H3K79 is generally linked to actively transcribed genes, whereas di- and tri-methylated H3K9, is considered a repressive mark (Martin and Zhang, 2005).

### 2.3. Nucleosome positioning

The position of nucleosomes relative to the start site of a gene represents another important regulator of transcription, and nucleosome positioning is significantly influenced by both DNA methylation and histone modifications. In most of the cases a nucleosome-free region upstream of the transcription start site is found in active genes, allowing access by the transcriptional machinery. However, nucleosomes can act as a barrier to transcription for some other genes and must be displaced for gene activation to occur (Li et al., 2007).

### 2.4. RNA mediated epigenetic mechanisms

Increasing evidence supports a role for non-coding RNAs in epigenetics. MicroRNAs (miRNAs) are a group of small noncoding RNAs that bind to the 3′ untranslated region (3′-UTR) of target mRNAs and mediate their post-transcriptional regulation leading to either degradation or translational inhibition, depending on the degree of sequence complementarity (Fig. 1). A complicated network of feedback between miRNAs and other epigenetic pathways appears to form an epigenetics-miRNA regulatory circuit, and to organize the whole gene expression profile (Sato et al., 2011). Also long non-coding RNAs (lncRNAs) participate in targeted gene silencing through chromatin remodeling, nuclear reorganization, formation of a silencing domain and precise control over the entry of genes into silent compartments (Saxena and Carninci, 2011).

### 2.5. Epigenetic mechanisms in physiology and pathology

Epigenetic mechanisms have several physiological roles. During development, germline DNA methylation is erased in the blastocyst, and a bimodal pattern is established anew at the time of implantation when the entire genome gets methylated while CpG islands are protected. This brings about global repression and allows housekeeping genes to be expressed in all cells of the body. Postimplantation development is characterized by stage- and tissue-specific changes in methylation that ultimately mold the epigenetic patterns that define each individual cell type (Cedar and Bergman, 2012). DNA methylation has been traditionally viewed as a highly stable epigenetic mark in post-mitotic cells, however increasing evidence suggests that changes in DNA methylation occur with aging, might be involved in several human diseases, characterize cancer cells, and are also fundamental for memory formation (Guo et al., 2011b; Rodríguez-Rodero et al., 2010; Sultan and Day, 2011; You and Jones, 2012). For example, postnatal brains appear to exhibit stimulus-induced methylation changes (Guo et al., 2011b), and there is consensus indicating that DNA methylation is dynamically regulated in the adult central nervous system in

response to experience and that DNA methylation may work in concert with histone tail modifications in memory formation (Sultan and Day, 2011). As a result, increasing evidence supports a role for epigenetics in autism and other neurodevelopmental disorders (Miyake et al., 2012), as well as in the pathogenesis of age-related neurodegenerative diseases (Migliore and Coppedè, 2009). Cancer cells also exhibit peculiar epigenetic patterns. For example, by the epigenetic point of view colorectal cancer can be divided into CIMP+ (CpG island methylator phenotype positive: display extensive levels of methylated genes) and non-CIMP tumors (Migliore et al., 2011). Epigenetic changes have a role also in several other human diseases, such as obesity, autoimmune disorders, and many others (Rhee et al., 2012; Rodriguez-Cortez et al., 2011). Given the increasing evidence for a contribution of epigenetics to human diseases, current research is focused on the development of the so called "epigenetic drugs" *i.e.* molecules with epigenetic properties to be used to counteract epigenetic changes occurring with aging, cancer, and other diseases (Schneider-Stock et al., 2012; Song et al., 2011). Moreover "environmental epigenetics" is gathering increasing attention from the scientific community. Several environmental agents are known to posses epigenetic properties and research interest is focused on the understanding of their biological consequences when interacting with cells of living organisms (Feil and Fraga, 2012; Hou et al., 2012).

## 3. Methods to detect epigenetic mechanisms

### 3.1. Gene-specific and genome-wide DNA methylation analysis

The earliest studies of methylation of specific sequences were almost entirely based on the use of methylation-sensitive and insensitive enzymes (Cedar et al., 1979) even though these approaches have some drawbacks, from incomplete DNA digestion to the limitation of the endonuclease cleavage sites. A major breakthrough in the analysis of DNA methylation occurred with the introduction of genomic DNA treatment with sodium bisulfite (Clark et al., 1994), that results in deamination of cytosines to uracils, while 5-methylcytosines are not converted (Taylor et al., 2007). Thus, during subsequent PCR amplification, uracil residues are replicated as thymine and 5-methylcytosine residues are replicated as cytosines. The reaction enables to distinguish methylated DNA from unmethylated DNA, and its combination with other methods allows defining the methylation pattern of a target sequence. All bisulfite-associated metodologies require PCR amplification of the transformed DNA and are based mainly on two different strategies in the design of primers for such reactions: methylation-specific PCR (MSP) primers that include several CpG sites toward the 3′-end and are designed for the amplification of a methylated template only, and methylation-independent PCR (MIP) primers that are independent by methylation and are designed for proportional amplification of methylated and unmethylated DNA.

Genomic sequencing is considered the gold standard method for DNA-bisulfite treated methylation analysis, as it determines the methylation state of each cytosine of the target sequence. After the modification of DNA, the fragment of interest is amplified by PCR using MIP, and PCR products can be sequenced directly or as single clones. Sequencing of cloned PCR products provides information on individual molecules, whereas direct sequencing provides an estimate of the average methylation status of each CpG site in all of the molecules. Unfortunately, sequencing of single clones is too time-consuming and expensive to be used in routine clinical settings (Mikeska et al., 2007).

The pyrosequencing is a widely used method which differs from Sanger sequencing, since it relies on the detection of pyrophosphate release on nucleotide incorporation, rather than chain termination with dideoxynucleotides (Tost et al., 2003). Pyrosequencing is usually carried out with MIP primers and allows to obtain quantitative data on individual CpG sites. This method is more sensitive and accurate respect to classical DNA sequencing since the analysis is performed by real-time sequencing.

Other MIP based methods include combined bisulfite restriction analysis (COBRA) (Xiong and Laird, 1997) and methods based on the use of melting curves analysis, which are able to distinguish methylated and unmethylated DNA template, since the higher CpG content in methylated sequences is more resistant to melting respect to unmethylated ones (Worm et al., 2001). The combination of this approach with High Resolution Melting (HRM) technology led to the development of methylation sensitive-HRM (MS-HRM), a very sensitive and specific method for the detection of methylation, which is less expensive compared to sequencing based approaches (Wojdacz and Dobrovic, 2007). Pyrosequencing provides information about each CpG sites, while MS-HRM gives the mean of CpG dinucleotides methylation; moreover our recent findings show a good correlation between the data obtained by means of both techniques, indicating their strong reproducibility and sensitivity. However depending on the study purpose, MS-HRM remains the favorite method since its time and cost effectiveness (Migheli et al., in press). A mass spectrometric sensitive approach has been also developed (Ehrich et al., 2005), but it is expensive and the complexity of the methodology can make it challenging to use.

Certainly the most widely used and less expensive method to detect CpG-island methylation is MSP (Herman et al., 1996). In the traditional MSP methodology, a second set of primers is often designed (in addition to the MSP primers) for the amplification of unmethylated DNA. Gel electrophoresis is used for detecting the PCR products, and in situations in which both unmethylated DNA and methylated DNA are present, a comparison of band intensity allows an estimation of relative methylation levels. Despite its wide use MSP is prone to give false positives, if primers are badly designed; further MSP is very sensitive but is not quantitative. Quantitative versions of MSP are MethylLight (Eads et al., 2000), which uses a fluorogenic probe hybridization or fluorescent dyes that intercalate dsDNA (Chu et al., 2002), Sensitive Melting Analysis after Real-Time MSP (SMART-MSP) methodology (Kristensen et al., 2008) that is based on the HRM technology (Kristensen and Hansen, 2009) and Methylation-Specific Fluorescent Amplicon Generation (MS-FLAG) in which the fluorescent signal is created by cleavage of quenched fluorophores at the 5′-end of double-stranded PCR products by the thermostable endonuclease *Psp*GI (Bonanno et al., 2007).

A nanotechnology variant of MSP, that enables the detection of methylation and its changes in a sensitive and quantifiable manner, is Methylation Specific quantum dot Fluorescence Resonance Energy Transfer (MS-qFRET), that requires the use of biotinylated methylation-specific primers, for post-PCR conjugation to quantum dots. The quantum dot nanoassay is strengthened through signal enhancement from multiple DNA targets that bind to a single quantum dot (Bailey et al., 2010).

Microarray-based DNA methylation profiling technologies have recently been developed to enable investigations of the methylation status for a large number of genes or the entire genome. The development of the Infinium Methylation assay by application of the Infinium® (East Sussex, UK) assay and BeadArray™ technology makes it possible to get a genome-wide high-throughput quantitative methylation profile of the human genome (Bibikova et al., 2009). The Infinium platforms incorporate a whole-genome amplification step after bisulfite conversion, which is followed by fragmentation and hybridization of the sample to methylation-specific DNA oligomers that are linked to individual bead types. Each bead type corresponds to a specific DNA CpG site and

methylation state and the percentage of methylation of a given cytosine is reported as a β-value that corresponds to the ratio of the methylated signal over the sum of the methylated and unmethylated signals (Laird, 2010). The recently developed Infinium HumanMethylation450 BeadChip® (Infinium Methylation 450K; Illumina, Inc., CA, USA) gives information on 485,000 methylation sites per sample at single-nucleotide resolution and it covers 96% of the CpG islands. This is a major extension of the previous Infinium HumanMethylation27. Infinium Methylation 450K includes multiple shores of the CpG islands as well as CpG sites located far from islands and intergenic regions, which may be important considering recent data that suggests that locations beyond CpG islands promoter methylation could be important as well (Ndlovu et al., 2011).

In summary, a large number of methodologies are available for the analysis of DNA methylation and the choose of one or another method determines the kind of information obtained, such as qualitative, quantitative and resolution level.

### 3.2. Histone tail modification analyses

In the past few years several techniques to study histone tail modifications have been developed. Protein microsequencing provides accurate sequence information and has demonstrated success in the identification of histone modifications but it requires a large amount of purified samples and only 20–25 residues can be routinely characterized (Su et al., 2007).

Chromatin immunoprecipitation (ChIP) is a widely used technique to detect interactions between DNA and proteins, in which chromatin is immunoprecipitated with antibody against a transcription factor, a chromatin-associated protein, or a modified histone. There are some variants of ChIP. Genomic sequences associated with the precipitated protein can be identified by polymerase chain reactions (ChIP-PCR), cloning and sequencing, high-throughput sequencing (ChIP-seq), or hybridization to microarray, that permits global scale analysis (ChIP-chip). The amount of protein binding to a specific region of DNA can be accurately and rapidly determined by quantitative-PCR in combination with ChIP (Irvine et al., 2011).

An intriguingly method recently developed, derives by the combination of ChIP and bisulfite methylation sequencing analysis (ChIP-BMS) (Li and Tollefsbol, 2011). This new technique detects the methylation status of ChIP DNA pulled-down by a specific antibody. ChIP-BMS could provide an excellent tool to investigate the interaction between histone modification and DNA methylation, transcription factor binding and methylation of recognition sites.

A different technique for the detection of protein–DNA interactions uses the marking of protein-binding sites by DNA adenine-methyltransferase (Dam). This technique, named Dam Identification (DamID), does not involve cross-linking or require antibodies or purification of protein–DNA complexes (van Steensel et al., 2001). DamID is an alternative method for mapping chromatin-associated proteins or transcription factors in which the protein of interest is fused to a DNA adenine methylase, and its DNA targets identified by restriction with adenine methylation-sensitive enzymes.

In the hydroxyapatite chromatography (HAP) technique the separation is based on the binding of hydroxyapatite to DNA in chromatin. Proteins are selectively eluted from the immobilized chromatin with NaCl in phosphate buffer. The HAP dissociation method enables chromosomal proteins to be fractionated with high recovery and without nucleic acid contamination. HAP is suitable for large-scale preparation of histones, nonhistone proteins, histone oligmers and/or DNA (Su et al., 2007).

Mass spectrometry (MS) is a physicochemical analysis technique that determines the mass-to-charge ratios of gas-phase ions and has quickly been accepted as a versatile tool to achieve insights into chromatin biology and epigenetics. A series of mass spectrometry-based technologies have been dedicated to the characterization and quantitation of different histone forms (Bonaldi et al., 2004).

Many proteins interact with RNA to modulate RNA-based epigenetic processes. RNA immunoprecipitation technique (RIP) is similar to ChIP assays. The principal difference between the two techniques is that DNA is enzymatically removed and RNA that is bound to the proteins captured by the antibody and then immunoprecipitated. The uses of the RIP technique may be help to understand the role of RNA in epigenetic processes (Selth et al., 2011).

### 3.3. MiRNA expression profiling techniques

Several approaches have been developed to evaluate miRNA expression profiles. By means of microarray analysis, that needs the use of probes for miRNA sequence, it is possible to analyze a large numbers of miRNAs and their regulation. Recently, next generation sequencing platforms became available for the sequencing of small RNA molecules, including miRNAs with the opportunity to discover novel miRNAs. The data obtained by these techniques need to be confirmed by miRNA-specific approaches to determine their accuracy. The most commonly used methods to detect specific miRNAs are real-time PCR analysis and Northern blotting analysis (van Rooij, 2011).

## 4. Epigenetic changes due to exposure to nano-sized materials

### 4.1. Airborne fine and ultrafine particles

For achieving knowledge on possible epigenetic effects caused by nanoparticles, one should take into account the literature available for ambient airborne nanoparticles. Ambient particulate matter (PM) is a complex mixture and the largest single source is most often traffic. PM is often divided into fractions depending on size. PM10 is basically the fraction of particles with an aerodynamic diameter $\leq$10 μm, the fine particle fraction is often measured as PM2.5, *i.e.* aerodynamic diameter $\leq$2.5 μm and the ultrafine fraction, PM0.1, is the smallest fraction with a diameter $\leq$0.1 μm. It is today well known that exposure to ambient particles is associated to different diseases such as cancer as well as respiratory- and cardiovascular diseases (Brook et al., 2010; Turner et al., 2011). One class of particles that is well known to cause adverse health effects is the diesel exhaust particles (DEP). These particles are often in the size of fine and ultrafine and they consist of an elemental carbon core to which various chemicals such as polyaromatic hydrocarbons (PAHs) and different metals are attached. Diesel exhaust was recently classified by IARC as carcinogenic to humans, and cardiovascular effects have been observed in controlled exposure studies on humans (Mills et al., 2011). Furthermore, several studies have revealed adjuvant effects on development and intensity of allergic inflammation, and it seems like small particles are more potent (Nygaard et al., 2004). This part of the review will summarize epigenetic effects observed after exposure to airborne fine and ultrafine particles (Table 1).

#### 4.1.1. DNA methylation

There is some evidence from human population studies suggesting that exposure to particles can have an impact on DNA methylation. Several studies suggest for example that methylation of long interspersed nuclear element-1 (LINE-1) repeated elements is decreased following exposure to particles. Baccarelli

**Table 1**
Studies on epigenetic effects of fine and ultrafine air pollution particles.

| Study model | Particle | Epigenetic effect | Reference |
|---|---|---|---|
| *DNA methylation* | | | |
| Blood cells *(Normative Aging Study)* | $PM_{2.5}$, black carbon | PM2.5 and black carbon associated with hypomethylation of LINE1 | Baccarelli et al. (2009) |
| Blood cells *(Normative Aging Study)* | $PM_{2.5}$, black carbon | Prolonged exposure to black carbon associated with hypomethylation of LINE1 and Alu | Madrigano et al. (2011) |
| Blood cells *(Normative Aging Study)* | $PM_{2.5}$, particle number, black carbon | Effect from air pollution (inflammation, coagulation, *etc.*) was stronger among subjects having higher Alu, but lower LINE-1, tissue factor (F3), or Toll-like receptor 2 (TLR-2) methylation status | Bind et al. (2012) |
| Blood cells *(Steel plant workers)* | $PM_{10}$, metals | PM10 associated with lower LINE1 and Alu methylation. *iNOS* methylation was significantly lower in postexposure blood samples (after 3 working days) compared with baseline | Tarantini et al. (2009) |
| Buccal cells *(Children's Health Study)* | $PM_{2.5}$ | Increased 7-day average PM2.5 exposure was associated with lower *iNOS* methylation | Salam et al. (2012) |
| Blood cells | Air pollution, $PM_{2.5}$, $PM_{10}$ | Increased exposure to ambient air pollution was associated with hypermethylation of the *Foxp3* locus | Nadeau et al. (2010) |
| Blood cells *(Steel plant workers)* | $PM_{10}$, $PM_1$, various metals | Promoter DNA methylation levels of *APC* and *p16* were higher in post-exposure samples compared to the levels in baseline samples. Mean levels of *p53* or *RASSF1A* promoter methylation was decreased | Hou et al. (2011) |
| C57BL/CBA mice *(Sperm)* | Air pollution particles near steel mill and highway | Sperm DNA was hypermethylated in mice breathing air particles when compared to HEPA-filtered air, and this change persisted following removal from the environmental exposure | Yauk et al. (2008) |
| BALB/c mice *(CD4+ cells)* | DEP | Diesel particle exposure resulted in hypermethylation of the *IFNG* promotor and hypomethylation of *IL4* promoter in CD+ cells | Liu et al. (2008) |
| Mice and cultured lung cells | $PM_{2.5}$ | PM 2.5 led to increase expression of the DNA methyltransferase 1 (*DNMT1*), and methylation of the *p16* promoter in mice and cells. | Soberanes et al. (2012) |
| *Histone modifications* | | | |
| Blood cells *(Steel plant workers)* | $PM_{10}$, $PM_1$, various metals | H3K4me2 and H3K9ac increased in association with years of employment in the steel plant. No clear relation to exposure to total mass of PM10 or PM1 but to inhalable nickel and arsenic. | Cantone et al. (2011) |
| A549 cell line | $PM_{10}$ | PM10 induced histone H4 acetylation at the *IL8* promoter as well as increased *IL8* expression. | Gilmour et al. (2003) |
| BEAS-2B cells | DEP | Diesel particle exposure led to increased histone H4 acetylation at the *COX2* promoter as well as increased *COX2* expression. | Cao et al. (2007) |
| *miRNA expression* | | | |
| Human primary bronchial epithelial cells | DEP | Diesel particle exposure led to changes in miRNA expression; miR-513, miR-494 and miR-923 were up-regulated whereas miR-96 was down-regulated | Jardim et al. (2009) |

et al. (2009) found that exposure to particles generated from traffic (black carbon) was associated with decreased methylation in LINE-1 in blood samples from elderly participants. These effects were observed within 7 days of the exposure measurement. In a similar study using the same cohort, Madrigano et al. (2011) also showed an association between decreased methylation in LINE-1 and exposure to black carbon. This study instead investigated more prolonged periods of exposure, and effects were generally observed 28–90 days after exposure. Using the same cohort, a recent study (Bind et al., 2012) investigated first of all if exposure to air pollution caused changes in blood markers of coagulation, inflammation and endothelial function (fibrinogen, C-reactive protein, intercellular adhesion molecule-1 (ICAM-1), and vascular cell adhesion molecule-1 (VCAM-1). Secondly, they investigated whether changes were dependent on the methylation status of DNA. They found that effect from air pollution was stronger among subjects having higher Alu, but lower LINE-1, tissue factor (*F3*), or Toll-like receptor 2 (*TLR-2*) methylation status. Thus, the results imply that DNA methylation may play a role in inflammatory gene expression and the risk for cardiovascular disease.

One animal study (Yauk et al., 2008) investigated changes in the global methylation following exposure to air pollution particles. C57BL/CBA mice were exposed *in situ* to ambient air near two integrated steel mills and a major highway, and control mice were breathing the same air that had been filtered using a HEPA filter. It was found that the mice with particle exposure had a 1.6-fold increase in sperm mutation frequency, no increase in bulky DNA adducts but an increase in DNA strand breaks. Furthermore, sperm DNA was hypermethylated in mice breathing ambient relative to HEPA-filtered air, and this change persisted following removal from the environmental exposure. The authors discuss that DNMTs are up-regulated during DNA damage and bind with high affinity to many DNA lesions (James et al., 2003) and thus, elevated DNA damage and subsequent up-regulation of DNMTs may lead to hypermethylation over time. The consequence of this increased methylation for the offspring is however unknown.

There are also some studies that investigated changes in methylation in specific genes. In blood leukocytes from workers in an electric furnace steel plant exposed to PM10, Tarantini et al. (2009) found that *iNOS* (inducible nitric oxide synthase) methylation was significantly lower in postexposure blood samples (after 3 working days) compared with baseline. In general, the authors discuss that changes in methylation is linked to cellular stress and inflammation. Another recent study also focused on the methylation status of *iNOS* and the relation between exposure to ambient PM2.5 in children (Salam et al., 2012). It was found that increased 7-day

average PM2.5 exposure was associated with lower *iNOS* methylation. Although exposure to PM10 was associated with lower *iNOS* promoter methylation the association did not remain statistically significant after adjusting for potential confounders. Exposures to $O_3$ and $NO_2$ were not either significantly associated with *iNOS* promoter methylation. Although *iNOS* methylation was associated with lower levels of exhaled nitric oxide, the association was not statistically significant. In another study on children, the authors investigated whether ambient air pollution can impair regulatory T-cell *via* hypermethylation of the *Foxp3* gene, a gene important in Treg-cell development and maturation (Nadeau et al., 2010). Indeed they found that increased exposure to ambient air pollution was associated with hypermethylation of the *Foxp3* locus, and the authors concluded that this leads to impairment of Treg-cell function and increasing asthma morbidity. Thus, air pollution seems to play a role in mediating epigenetic changes in Treg cells, which may worsen asthma by an immune mechanism. Furthermore, Liu et al. (2008) exposed BALB/c mice to DEP for three weeks while at the same time they underwent intranasal sensitization to *Aspergillus fumigatus.* This exposure induced hypermethylation of the *IFNG* promoter and hypomethylation of *IL-4* in CD4+ T cells among mice sensitized to the fungus allergen *A. Fumigatus.* The altered methylation of promoters of both genes correlated significantly with changes in IgE levels.

Except from genes involved in inflammation, changes in DNA methylation of tumor suppressor genes represent an important mechanism for cancer development. In a recent study, Hou et al. (2011) investigated whether exposure to PM, as well as different metal components, was associated with DNA methylation in 4 tumor suppressor genes (*APC*, *p16*, *p53* and *RASSF1A*) in blood leucocytes. These genes are involved in cell-cycle control (*p16*), invasion and metastasis (*APC*) apoptosis and cell cycle control (*p53*) and Ras signaling (*RASSF1A*). Blood samples were obtained on the first (baseline) and 4th day (post-exposure) of the same work week. It was found that mean promoter DNA methylation levels of *APC* and *p16* were significantly higher in post-exposure samples compared to the levels in baseline samples, but in contrast, the mean levels of *p53* or *RASSF1A* promoter methylation was decreased in the post-exposure samples (Hou et al., 2011). The authors discuss the potential use of altered DNA methylation in certain genes as biomarkers to detect biological alterations after PM exposure. The biological significance of findings like this is however still an open question.

Hypermethylation of the *p16* promoter was also investigated in a recent interesting study using mice as well as primary murine alveolar epithelial cells in culture (Soberanes et al., 2012). The mice were exposed to concentrated ambient PM2.5 *via* inhalation (8 h daily for 3 weeks) and the epithelial cells were exposed to 5 $\mu g/cm^2$ PM2.5 daily for 10 days. In both mice and alveolar epithelial cells, PM exposure increased ROS production as well as expression of the DNA methyltransferase 1 (*DNMT1*), and methylation of the *p16* promoter. The underlying mechanisms were studied by using the epithelial cells and it was found that increased transcription of *DNMT1* and methylation of the *p16* promoter were inhibited by a mitochondrially targeted antioxidant and a JNK (c-jun-n-terminal protein kinase, a member of the mitogen activated protein kinase family) inhibitor, suggesting the importance of mitochondria-generated ROS for the hypermethylation of the *p16* promoter.

#### 4.1.2. Histone modifications

Some *in vitro* studies suggest that histone modifications can be important in mediating the inflammatory response from particles. Gilmour and colleagues showed that PM10 increased IL-8 protein release from A549 cells after 24 h and furthermore that the release was enhanced by histone deacetylase inhibition (by using co-treatment with trichostatin A). An increased HAT activity was also observed as well as an increase of the level of acetylated histone 4 (H4). The enhanced H4 acetylation appeared to be mediated by oxidative stress since less acetylation was observed during co-treatment with N-acetyl-L-cysteine (NAC). Acetylation of H4 mediated by PM10 was associated with the promoter region of the *IL-8* gene (Gilmour et al., 2003). Similarly, Cao et al. (2007) showed that DEP can induce the expression of the *COX-2* gene in human bronchial epithelial cell line (BEAS-2B) and at the same time recruitment of histone acetyltransferase (HAT) p300 to the promoter of the *COX-2* gene was observed, which suggests that acetylation is important in regulating the *COX-2* gene. In addition, DEP caused degradation of histone deacetylase 1 (HDAC1).

In a recent study involving 63 steel workers, Cantone et al. (2011) investigated whether particles (PM10 and PM1), as well as more specifically some metal components of PM caused changes in activating histone modifications. The levels of histone 3 lysine 4 dimethylation (H3K4me2) and histone 3 lysine 9 acetylation (H3K9ac) on histones from blood leukocytes were examined and related to total PM and to the inhalable metal components; aluminum, manganese, nickel, zinc, arsenic, lead and iron. Both H3K4me2 and H3K9ac increased in association with years of employment in the plant and an association was found to exposure to inhalable nickel and arsenic (Cantone et al., 2011). There was however no clear relation to exposure to total mass of PM10 or PM1.

#### 4.1.3. miRNA expression

Changes in miRNA expression following exposure of lung cells to DEP *in vitro* were investigated by Jardim et al. (2009). Primary human bronchial epithelial cells were grown at air–liquid interface and following differentiation they were exposed to 10 $\mu g/cm^2$ DEP for 24 h. The miRNA expression was then assessed using microarray profile analysis and quantitative real-time polymerase chain reaction. It was found that 197 of 313 detectable miRNAs (62.9%) were either up-regulated or down-regulated by 1.5-fold. Especially miR-513, miR-494 and miR-923 were confirmed to be significantly up-regulated whereas miR-96 was down-regulated. These changes may contribute to the inflammatory response and could be part of the mechanism to why DEP cause diseases.

### 4.2. Engineered nanomaterials (ENMs)

Initial studies attempting to evaluate the effects of engineered nanoparticles have focused on their capabilities to induce changes in gene expression profiles, without questioning of whether or not this was due to epigenetic modifications. More recently, those studies have been paralleled by others aimed at investigating the epigenetic properties of those compounds, *i.e.* their ability to induce changes in DNA methylation, chromatin folding or miRNA expression. Gene expression studies have been so far performed with a number of nanoparticles. For example, manganese, silver, and copper NPs induced changes in the expression of dopaminergic system-related genes in PC12 cells (Wang et al., 2009), and alteration of the genomic profile was evidenced both *in vivo* and *in vitro* after treatments with metallic and metal oxide-based NPs, fullerens, and luminescent particles (Eom and Choi, 2009; Huang et al., 2010; Okuda-Shimazaki et al., 2010; Papis et al., 2007; Rahman et al., 2009). In silver nanoparticles (AgNP) exposed cells (human lung cells and human brain cancer cells) it was observed an up-regulation of many DNA damage response genes such as *Gadd 45* and down regulation of genes necessary for cell cycle progression (*cyclin B* and *cyclin E*) and involved in DNA damage repair (*XRCC1* and *3*, *FEN1*, *RAD51C*, *RPA1*) (Asharani et al., 2012). In human lung epithelial cell line (A549) and HeLa cells AgNPs were able to alter the regulation of more than 1000 genes including

members of the metallothionein, heat shock protein and histone families (Foldbjerg et al., 2012; Xu et al., 2012). Silica nanoparticles ($SiO_2$ NPs) in human endothelial cells strongly induced heme oxygenase-1 (*HO-1*) mRNA, a marker for particle-induced oxidative stress (Napierska et al., 2012). In human keratinocyte cell line (HaCaT) gold nanoparticles (AuNPs) caused significant ROS production and up-regulated several genes involved in cellular stress and toxicity (Schaeublin et al., 2012). ZnO NPs induced gene transcription changes, in RKO and CaCo-2 cells affecting genes involved in metal metabolism, chaperonin proteins, and protein folding (Moos et al., 2011) and up-regulated and down-regulated *c-Myc* and *Bcl-2* genes respectively in human bronchial epithelial (16HBE) cells (Fu et al., 2012). Ceria NP ($CeO_2$ NPs) were found to have different modes of action according to the cell type, including A549 or BEAS-2B cells. However elevated reactive oxygen species levels, increased lipid peroxidation, and membrane damage, reduced antioxidant levels as well the production of 8-oxoguanine after prolonged exposure are all evidence of oxidative stress caused by $CeO_2$ NP (Hirst et al., 2009; Lin et al., 2006). Oxidative stress-related genes were induced by 40 μg/ml of 30 nm particles, while altered gene expression and modest mRNA upregulation of the inflammatory markers IL8 and MCP-1 were found in other studies (Gojova et al., 2009; Park et al., 2008; Rothen-Rutishauser et al., 2009). In the subsequent subparagraphs we review the few studies performed so far to evaluate epigenetic properties of ENMs (Table 2).

#### 4.2.1. DNA methylation

Concerning epigenetic properties of engineered NPs, $SiO_2$ NPs were found to induce global DNA hypomethylation in HaCaT cells in association with *DNMT1* and *DNMT3a* gene expression decrements. The mRNA expression of *MBD2* changed in a trend similar to that of *DNMT1* and *DNMT3a* and alterations at the protein level were similar to those at the mRNA level (Gong et al., 2010). More recently, the same group observed that $SiO_2$ NPs were able to induce *PARP-1* hypermethylation in HaCaT cell line with a simultaneously decrease of *PARP-1* gene expression and its protein levels (Gong et al., 2012). The *PARP-1* gene encodes for the DNA repair protein poly-ADP-ribose polymerase-1 (PARP-1), a zinc-finger DNA binding protein that is activated by single- or double-strand breaks in DNA. The primary function of PARP-1 is in DNA repair processes through the detection of DNA damage and the prevention of chromatid exchanges. PARP-1 poly-ADP-ribosylates several proteins involved in DNA repair including histones, thus inducing local relaxation of the chromatin structure and facilitating the access of repair proteins to damaged DNA. However, PARP-1 activation can also result in cell death. Indeed cells with extensive DNA damage have a widespread PARP-1 activation with a subsequent depletion of its substrate $NAD^+$, and then ATP, leading to energy failure and cell death (Kauppinen and Swanson, 2007).

#### 4.2.2. Histone modifications

Cadmium telluride quantum dots (CdTe QDs) induced a global hypoacetylation in human breast adenocarcinoma cells (MCF-7) (Choi et al., 2008). Moreover mRNA levels of two genes involved in preventing cell death, *cIAP-1* (Inhibitor of apoptosis) and *Hsp70* (Heat shock protein 70), were significantly reduced in CdTe QDs-treated while *GPx* (glutathione peroxidase) mRNA expression was completely suppressed. Conversely CdTe QDs induced an increase in the mRNA levels of p53-target genes *Bax*, *Puma*, and *Noxa* (Choi et al., 2008).

#### 4.2.3. miRNA expression

Titanium nanoparticles ($TiO_2$ NPs) showed epigenetic properties in mice (Halappanavar et al., 2011). After treatment of female C57BL/6BomTac mice with $TiO_2$ NPs, the expression profiles of 53 genes had fold changes higher than 1.5 in lung of exposed *versus* control mice (50 resulted upregulated and 3 downregulated), among which genes involved in acute phase response, cytokine and chemokine signaling pathways. However, changes in gene expression did not correlate with those in protein synthesis, suggesting the existence of post-transcriptional mRNA regulatory mechanisms. Indeed, several miRNAs were significantly altered by $TiO_2$ NPs exposure. In particular the researchers observed upregulation of miR-449a (6-fold), miR-1 (2.6-fold), and miR-135b (60-fold), suggesting that those miRNAs may be involved in the regulation of mRNA translation into proteins (Halappanavar et al., 2011). An increased expression of tumor necrosis factor (*TNF-α*) and neutrophil attracting chemokines (*CXCL1* and *CXCL8*) mRNAs was observed in alveolar macrophages of mice and in human cell cultures following exposure to $SiO_2$-coated rutile $TiO_2$ NPs, while uncoated $TiO_2$ NPs showed no effect on gene expression (Rossi et al., 2010).

AuNPs are one of the most versatile and widely researched materials for novel biomedical applications. However, the current knowledge in their toxicological profile is still incomplete. *In vitro* AuNPs were found to induce oxidative stress-mediated genomic instability in MRC-5 lung fibroblasts. This finding was related to the differential expression of proteins, in particular oxidative stress-related as well as proteins associated with cell cycle regulation, cytoskeleton and DNA repair (Li et al., 2011a). After the treatment with AuNPs, a dramatic phenotypic modification in the subsequent generations of *Drosophila* was observed, demonstrating their capability to induce mutagenic effects that may be transmitted to the descendants (Vecchio et al., 2012). After a single intravenous

**Table 2**
Studies on epigenetic effects of engineered nanoparticles.

| Study model | Nanoparticle | Epigenetic effect | Reference |
|---|---|---|---|
| *DNA methylation* | | | |
| HaCaT cell line | $SiO_2$ NPs | Global DNA hypomethylation and *DNMT1*, *DNMT3a* and *MBD2* mRNA repression | Gong et al. (2010) |
| HaCaT cell line | $SiO_2$ NPs | *PARP-1* hypermethylation and *PARP-1* mRNA repression | Gong et al. (2012) |
| *Histone modifications* | | | |
| MCF-7 | CdTe QDs | Global hypoacetylation | Choi et al. (2008) |
| *miRNA expression* | | | |
| C57BL/6BomTac mice | $TiO_2$ NPs | Upregulation of miR-449a (6 fold), miR-1 (2.6 fold), and miR-135b (60 fold) | Halappanavar et al. (2011) |
| MRC5 cell line | Au NPs | Upregulation of miR155 with concomitant down-regulation of *PROS1* gene; chromatin condensation | Ng et al. (2011) |
| NIH/3T3 cells | MW-CNTs | Deregulation of miRNA expression | Li et al. (2011b) |
| NIH/3T3 cells | CdTe QDs | Global alteration miRNAs expression patterns | Li et al. (2011c) |
| *Nicotiana tabacum* | $Al_2O_3$ NPs | Increased expression of many miRNA such as miR395, miR397, miR398, and miR399 | Burklew et al. (2012) |

(i.v.) administration of PEG-coated AuNPs of different sizes, (4 and 100 nm) the liver tissue of BALB/c mice was analyzed for the gene expression profile. Commonly expressed genes were categorized as apoptosis, cell cycle, inflammation, and metabolic process (Cho et al., 2009). A significant biodistribution of Au was found occurring in rats 2 months after a single i.v. injection of AuNPs, accompanied by expression changes on genes related to detoxification, lipid metabolism, cell cycle, defence response, and circadian rhythm in liver and spleen (Balasubramanian et al., 2010). The epigenetic effects of AuNP were evaluated in MRC5 human fetal fibroblasts (Ng et al., 2011). 19 genes involved in different cellular pathways such as stress-responsive genes, genes that regulate cellular morphogenesis, blood coagulation, hemostasis, hydrolase activity, metal ion binding and sterol metabolism, were found to be differentially expressed with up-regulation of 9 genes and concomitant down-regulation of 10 genes in AuNP-treated cells. Gene expression alterations of interest were upregulation of microRNA 155 and concomitant down-regulation of *PROS1* gene. *PROS1* codes for a protein called protein S that is important for controlling blood clotting, acting as a cofactor of enzymes involved in this process. Silencing of miR-155 increased the expression of *PROS1* at mRNA and protein levels suggesting that miR-155 could regulate expression of the gene. The DNA methylation profile of *PROS1* was also evaluated and resulted unaffected by AuNP treatment. Chromatin reorganization was evaluated by means of transmission electron microscopy (TEM), that revealed the presence of chromatin condensation in AuNP-treated fibroblasts in comparison with untreated fibroblasts (Ng et al., 2011).

miRNAs expression profile in NIH/3T3 cells after exposure to $Fe_2O_3$ NPs, CdTe QDs and MW-CNTs was evaluated by Li et al. (2011b); the expression levels of many miRNAs were widely affected, and in particular MW-CNTs induced the higher miRNA expression deregulation. CdTe QDs also globally affected the expression patterns of miRNAs in NIH/3T3 cells (Li et al., 2011c).

Both porcine pulmonary artery endothelial cells and human umbilical vein endothelial cells showed increased mRNA and protein expression of endothelial cell adhesion molecules (VCAM-1, ICAM-1, and ELAM-1) after exposure to alumina nanoparticles (Oesterling et al., 2008). Little is still known regarding to epigenetic effects induced by those compounds. However, a recent study conducted on exposed tobacco plants (*Nicotiana tabacum*) showed that Al2O3 NPs had a negative effect on the growth and developmental of the plant seedlings with concomitant increase in expression of many miRNAs such as miR395, miR397, miR398, and miR399, that are involved in plant stress responses (Burklew et al., 2012).

#### 4.2.4. Other "epigenetic" effects

In 2010 Alpatova et al. evaluated the "epigenetic toxicity" of single-walled carbon nanotubes (SWCNTs) in WB-F344 rat liver epithelial cells (Alpatova et al., 2010). Trosko et al. (1998) defined "epigenetic toxicity" as a consequence of disruption in gap junction intercellular communication (GJIC) that secondarily would result in modulation of gene expression at the transcriptional, translational or post-translational levels. Alpatova and colleagues found that WB-F344 cells exposed to disperse SWCNTs retain normal intercellular communication irrespective of the applied treatment; however, in our opinion, these findings are not identifiable in a classical epigenetic context.

## 5. Concluding remarks

A growing body of evidence indicates that epigenetic modifications have a role in the onset of several human diseases, including among others cancer (Dawson and Kouzarides, 2012; Migliore et al., 2011), neurodegenerative diseases (Migliore and Coppedè, 2009), cardiovascular complications (Udali et al., 2012), autoimmune disorders (Rodriguez-Cortez et al., 2011), as well as behavioral disturbances and psychiatric disorders (Miyake et al., 2012). In addition, evidence is emerging of possible transgenerational effects following *in utero* exposures. For example, the offspring of female rats exposed to lead (Pb) during pregnancy, showed age-related neuropathological changes resembling those of Alzheimer's disease (AD) and changes of methylation of key AD genes (Basha et al., 2005).

Tables 1 and 2 summarize the epigenetic effects until now observed on nano-sized particles. Those studies highlight the potential of nano-sized compounds to induce global DNA methylation changes (LINE1 and Alu methylation), as well as changes of gene specific methylation patterns, including tumor suppressor genes (*APC*, *p16*, *RASSF1A*, *p53*), inflammatory genes (*iNOS*, *IFNG*, *IL4*), DNA repair genes (*PARP-1*), and impaired expression of genes involved in DNA methylation reactions (*DNMT1*, *DNMT3A*, *DNMT3B*, *MBD2*), all potentially involved in cancer development. Of particular interest is the study by Yauk et al. (2008) reporting changes in sperm DNA methylation of mice exposed to air pollution particles, since it shows that epigenetic changes due to the exposure to those compounds are not limited to the exposed individuals but might affect their germline cells and be transmitted to future generations. Exposure to nano-sized materials is also able to induce changes in the acetylation and methylation of the histone tails, thus rendering the chromatin structure more or less accessible to transcription and DNA repair complexes (Cantone et al., 2011; Choi et al., 2008; Gilmour et al., 2003). Furthermore, several investigators observed that the exposure to nano-sized materials induces either global or gene specific alteration of miRNA expression (Burklew et al., 2012; Jardim et al., 2009; Li et al., 2011b, 2011c), likely resulting in an impaired expression of all the genes whose expression is regulated by those miRNAs.

At present there is an awareness that understanding the true effect of environmental exposures on the human epigenome will require additional research with appropriate studies and application of novel technologies. Our current screening tests usually do not detect agents that act throughout epigenetic mechanisms. The aim of this review was to give also an overview of new experimental approaches that can be included in new assays.

The epigenetic mechanisms that are intensively investigated include (i) chromatin structure and histone modification that gate the access of transcriptional machinery to genes, (ii) non-coding RNAs activity including microRNA that regulate gene expression through altering chromatin configuration, inhibition of translation, and degradation of RNA and finally (iii) the epigenetic information encoded in the DNA methylation pattern. Since the biological significance of differentially methylated regions (*e.g.* promoter of genes but also CG islands of non coding regions) can vary depending on the functional role of the regions involved, whole genome technologies that allow examination of differentially methylated regions and also gene-specific approaches can offer complementary information. Moreover, it would be useful not only to employ assays able to detect agents that interfere with DNA methylation, but also to consider the possibility that they might act indirectly through affecting chromatin modification.

Given the widespread use and dispersion in the environment of nano-sized materials, at present and foreseeable in the near future, and in light of their potential epigenetic properties here reviewed, more attention should be paid to the possible outcomes in exposed individuals, as well as the possible induction of effects also in the offspring.

## Conflict of interest statement

The authors declare no conflict of interest with the study or preparation of the manuscript.

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