Journal of Molecular and Genetic Medicine

Yao and Costa, J Mol Genet Med 2013, 7:4
DOI: 10.4172/1747-0862.1000086

Review Article Open Access

# Genetic and Epigenetic Effects of Nanoparticles

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**Abstract**

Nanoparticles can occur naturally or be intentionally engineered. The field of nanotechnology has varied influence on industry and segments of our daily life. The health effects associated with human exposure to nanoparticles remains elusive and very little has been done with respect to investigating the genetic and epigenetic effects of nano-materials. This is especially concerning given their wide spread applications in the modern world. We reviewed recent findings of the genetic and epigenetic effects of several common nanoparticles that humans are exposed to. Problems and concerns existing in current nano-toxic studies are addressed and discussed.

## Introduction

Nanoparticles (NP) are particles with dimensions smaller than 100 nm [1,2]. Engineered NPs are widely used in cosmetics, clothing, sunscreen and food additives, etc. Current applications of nanotechnology have great influence on various industry and medical sectors. The global market for nanotechnology based manufactured goods is expected to be worth US$ 1.6 Trillion by certain estimations, representing a compound annual growth rate of around 50% during 2009-2013. Engineered NPs with a diameter of less than 100nm are classified as ultrafine particles. Ultrafine particles can occur naturally, or can be generated through combustion sources such as cooking, candle burning, and tobacco smoking, while engineered NPs are produced intentionally for industrial purposes and generally with greater consistency in size and chemistry. These particles readily travel throughout the body, having higher deposition rates in the lower respiratory tract [1,2].

Human exposure to ultrafine particle occurs mostly through ambient atmospheric exposure; therefore the respiratory tract is the preferred target for exposure [2,3]. Exposure to engineered NPs can happen through other pathways (inhalation, dermally, ocularly).The wide spread use and applications such as oral administration through food or drinking water, skin absorption through sunscreen and/or cosmetics application, injection through medical procedures presents a number of potential problems [4]. The fate and risk may differ through different exposure pathways. Most animal studies have found over 90% orally administrated engineered NPs were excreted through feces [5,6]. However, with their small sizes, the retention of NPs in the liver and kidney has been found [7,8]. The employment of nanoparticles in sunscreens has raised the question of whether these particles are photo-clastogenic. Intensive studies have led to controversial results, which will be discussed later.

Much effort has been devoted to understand the toxicity of ultrafine particles. Genetic and epigenetic effects are parts of the toxicity of NPs. The small size and large surface area facilitate the generation of free radicals, and the induction of oxidative stress [3,9,10]. Tissue culture analysis in animal models demonstrates that oxidative stress contributes significantly to the cytotoxicity and genotoxicity associated with NP exposure [2,11,12]. It has been found that lipid peroxidation and oxidative stress are the most important mechanisms of genotoxicity related to NP exposure [13]. An emerging area of concern, are the epigenetic effects of NPs and has attracted growing interests. The findings and their implications will be discussed later.

The biological impact and biokinetic distribution of NPs are affected by many parameters including size, chemical composition, surface structure, solubility, shape, and aggregation. These parameters can modify cellular uptake, translocation from exposed organs to the targeted sites and the severity of the tissue injury [2]. Therefore, *in vivo*/*in vitro* toxicity assays need to reflect effects on the exposed organs including lungs, skin, and mucus membranes. Additional focus needs to be given to target tissues and systems such as endothelium, blood cells, spleen, liver, nervous system, heart and kidney; most importantly, at a physiological relevant concentration of exposure.

The purpose of this minireview is to utilize several types of NPs as examples to survey the genetic and epigenetic effects of NPs exposure, and to address the importance of their physical /chemical features as well as bioavailability on these effects. Our review is limited to several examples and is no way comprehensive of all types of nanoparticles.

## The Genetic Effects of NPs

The genetic effects of NPs, by definition, include DNA damage, possibly leading to mutations, DNA strand breaks and chromosomal aberrations [14]. The mechanism of NPs genetic effects are as follows [15], (1) direct binding to the DNA: some NPs are capable of localizing within the nucleus, directly interacting with the DNA molecule [16,17]; (2) direct binding to DNA associated proteins: where the NPs do not physically interact with the DNA molecule, but with other cellular proteins such as those involved in the chromatin structure or DNA replication process; (3) indirect cellular responses: oxidative stress, inflammation and aberrant signaling activation [18,19].

Two examples, of NPs emitted from laser printers/photocopiers as well as titanium dioxide ($TiO_2$), are discussed as examples of genetic effects and to consider the importance of physical chemical features as well as bioavailability of NPs to their risk assessment.

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**Received** September 30, 2013; **Accepted** October 29, 2013; **Published** November 04, 2013

**Citation:** Yao Y, Costa M (2013) Genetic and Epigenetic Effects of Nanoparticles. J Mol Genet Med 7: 86. doi: 10.4172/1747-0862.1000086

## NP emitted unintentionally

An unintentional release of potentially dangerous particles can be caused by mechanical manipulation of earth or other processes such as drilling, sawing, or sanding, by abrasion during daily use, or by degradation of the matrix caused by aging or weathering including water absorption, oxidation, or exposure to UV light. One example, that sampled and examined NP using transmission electron microscope (TEM in a sanding mimic abrasion process) showed that free-standing Carbon Nano Tubes (CNTs) were released into the environment during this process [20]. NPs emitted unintentionally are complex non-homogenous in their chemical composition which increases the difficulty of studying their effects. Among some of the more environmentally relevant NPs of interest are ones emitted from printers and photocopiers. These particles have been studied intensively, and therefore their properties are better understood. Toners emit a mixture of organic compounds and inorganic metal oxide additives [21]. The organic fraction of these NPs are formed primarily from the condensation of semi-volatile organic compounds evaporated from the toner and possibly other paper constituents during the printing/photocopying process, and their fraction remains poorly characterized due to its diversity [2,6]. The inorganic fraction of airborne NPs varies with the toner formulation and may contain variable amounts of silicon (Si), sulphur (S), titanium (Ti), iron (Fe), chromium (Cr), nickel (Ni), zinc (Zn) and possibly other elements, most likely originating from the metal oxide additives in toners [21]. The major route of entry of airborne NPs is by inhalation [22,23].

A recent study which recruited young volunteers to spend time in busy photocopy centers (2-3 days a week, 6 hours a day) found higher levels of 8-OH-dG in their urine when compared to the days spent in printer-free environments [24], indicating that the elevated NP levels in these volunteers lead to a measurable level of oxidative stress and thereby modified their genetic material. A549 cells treated with NPs emitted from laser printers exhibited more micronuclei which resulted from DNA double strand break [21].

Various chromosomal aberrations have been found at significantly higher levels in a study of buccal epithelial cell and peripheral blood samples from males working with photocopying machines for more than a year when compared to their age matched unexposed controls [25]. Carbon NP and inflammation induced by carbon deposits might have caused these reported genetic effects [26]. A case report of, a female patient with weight loss and diarrhea after three years of exposed to laser printers in her office showed black material deposited in her submesothelial tissue and associated inflammatory reaction. A scanning electron microscopy study revealed that the submesothelial aggregates consisted of carbon NPs with sizes ranging from 31 to 67 nm [26].

Much effort is still needed to investigate other unintended NP emissions associated with our daily life. The respiratory tract is particularly susceptible to cellular assaults caused by inhaled NPs, which makes the unintended NP emission in workplaces and the daily environmental risk factor that may be detrimental to human health.

## $TiO_2$

Engineered titanium dioxide NPs are a widely used and through their design, exhibit properties that are genotoxic.

**Genetoxicicity originating from different physical chemical features of NPs:** Titanium dioxide is a poorly soluble particulate produced either in its anatase or rutile crystal form in industrial setting [27]. It is the most widely used white pigment in products such as paints, film, paper, food additives and cosmetics because of its brightness and high refractive index [28,29]. A comparative study using keratinocytes to investigate different crystalline phases of $TiO_2$ interaction with cells showed that the anatase phase, which is phagocytosed in small clusters and is lodged inside the mitochondria, is more effective in producing free radicals and thereby generating significantly greater amounts of oxidative stress than its rutile phase [30]. Other than the crystalline phases of $TiO_2$, the agglomeration and dispersion status of the NP can also modulate its genetic effects. A study using TK6 human lymphoblast cells and Cos-1 monkey kidney fibroblasts has shown that less stable dispersion may easily lead to larger agglomerates and thereby inducing DNA damage [31]. This DNA damage included strand breaks and oxidative damage which were analyzed by alkaline and FPG-modified comet assay [31-33].

**Genotoxic variability originating from different entry points:** As discussed above, the exposure route and translocation efficiency most certainly will affect the toxicity of the NP. The bright white color, ability to block UV light, and antimicrobial activity of $TiO_2$ NPs have made it a quite popular component in the food industry, in cosmetics and sunscreen manufacture, which make inhalation, ingestion and dermal exposure a common exposure route for humans [34-37]. The primary route of occupational exposure for $TiO_2$ NPs is inhalation [2]. Consumer inhalation is also possible during application of antibacterial spray containing $TiO_2$ NPs [38].Upon inhalation or instillation, small fractions of $TiO_2$ NPs are transported from the airway lumen to the blood circulation and reach extra pulmonary tissues such as liver and kidneys [39,40]. *In vitro* studies using cells from extra pulmonary tissues such as immortalized brain microglia (BV2), primarily cultured lymphocytes, and human B-cell lymphoblastoid cell line exhibited chromosome aberrations as well as oxidative DNA damage when treated with $TiO_2$ [12,41,42]. However, whether the genetic effects reported in these *in vitro* studies are applicable to other routes of entry will largely rely on the biokinetic distributions and whether there is a co-exposure to other pathogens [43].

**Oral administration:** The Food and Drug Administration (FDA) has allowed 1% by weight of $TiO_2$ as a food additive (FDA-21 CFR 73.575). Therefore, $TiO_2$ NP exposure from food-related ingestion is a highly relevant exposure route that needs further study. An *in vivo* study with 13 weeks of repeated oral administration of anatase 80:20 rutile $TiO_2$ NP to rats, up to 1041 mg/kg body weight, didn't find significant concentration dependent increase of $TiO_2$ in blood samples. The $TiO_2$ distribution to the liver, spleen, kidney, and brain was also minimal. No dose-response relationship was seen, meaning that the $TiO_2$ particles were not significantly absorbed and distributed [7]. This result indicates that gastric ducts will be the major tissues that will be affected by ingestion of $TiO_2$ NPs. $TiO_2$ NP have been shown to induce DNA strand breaks by comet assays in gastric epithelial cells [44]. However an *in vivo* murine model study has argued that other organs might be targets for $TiO_2$ NP genetic effects. Repeated oral administration of anatase75: 25rutile $TiO_2$ NP, up to 500 mg/kg body weight has led to increased DNA deletion frequency in fetuses after maternal exposure [37]. Nevertheless, a consistent concentration-dependent response of DNA strand breaks and chromosomal damage has been found in bone marrow and peripheral blood from exposed non-pregnant mice, as well [37]. The underlying mechanism driving these results remains elusive. The fact that neither of these studies employed organ deposition analysis as comparison with DNA damage tests has led to a limited interpretation of these data [7,37]. Whether the

*in vivo* DNA damage effects of $TiO_2$ were from an indirect mechanism or due to special preparation of $TiO_2$ samples remains unknown.

**Dermal administration:** Most of the dermal exposure studies have shown that $TiO_2$ NPs do not penetrate the stratum corneum, and only penetration into orifices of the pilosebaceous follicles was observed [45]. However, even with preferential distribution, the fact that $TiO_2$ NPs are widely used in sunscreens and cosmetics has raised the concern of photo-clastogenicity, given that sunscreens are always applied when there's UV exposure. It was reported that $TiO_2$ produced the OH radicals, $H_2O_2$ and $O_2$ under UV irradiation [46,47], while anatase $TiO_2$ NP lead to higher levels of OH radicals when compared to the rutile form, the OH radicals levels were correlated with the UVA dose [48,49].

In an early *in vitro* genotoxicity study, the single cell gel and chromosomal aberration assay showed that $TiO_2$ particles induced primary DNA damage and structural chromosome aberrations in cultured L5178Y mouse lymphoma cells when exposed to a UV spectrum similar to natural sun light. These genotoxic effects were dependent upon $TiO_2$ dose and solar light intensity. Gene mutations were not induced by photo excited $TiO_2$ particles in microbial or mammalian cell systems. Therefore, it was proposed that the DNA lesion catalyzed by photo excited $TiO_2$ particles resulted in chromosomal aberration rather than gene mutations [50].

Studies conducted afterwards disputed these findings by showing none of the eight different forms of uncoated, coated and doped $TiO_2$ NP was able to induce chromosome aberrations in Chinese hamster ovary cells, with or without the presence of simulated solar light [51]. The latter study argued that the previous L5178Y mouse lymphoma cell studies results of comet tail lengths were only evident at concentrations where cell survival was 70% or less, and increased chromosomal aberration frequencies occurred when cells were experiencing>50% cytotoxicity [51].

## The Epigenetic Effects of NPs

"Epi" means above, outer and over in Greek, therefore "epigenetic" literally means "above genetic" which are heritable changes in phenotypes or gene expression without a change of DNA sequences [52]. Epigenetic effects involve inducing alterations in DNA methylation patterns, posttranslational modification of histones tails, chromatin remodeling and non-coding RNA. If these changes persist through cell division, heritable altered gene expression pattern will occur [52].

Several NPs have shown epigenetic effects and may lead to health risks to exposed cells which fit within the scope of this mini-review. It goes without saying that engineered NPs that are designed for epigenetic therapy purpose such as NPs formed by histone deacetylase inhibitors [53] and others [54-56] will not be discussed here.

### DNA methylation

Methylation on cytosines and their subsequent interaction with methyl-CpG binding proteins (MBDs) act as regulatory marks to induce chromatin conformational change and inhibit the access of the transcriptional machinery, thus altering gene expression [57]. DNA methyl transferases (DNMTs) catalyze the transfer of a methyl group to cytosine [58]. In mammals DNMT1 is primarily involved in the maintenance of DNA methylation patterns during development and cell division, where as DNMT3a and DNMT3b are the *de novo* methyl transferases and establish DNA methylation patterns during early development [59]. DNMT3L induces *de novo* DNA methylation by recruitment or activation of DNMT3a, while DNMT2 is primarily involved in the methylation of transfer RNA (tRNA) [59].

Promoter hypermethylation is commonly associated with gene silencing [60] with a few exceptions [61] where intragenic methylation might also have a role in regulating gene expression [62,63].

Silica nano particles ($SiO_2$ NP) are highly stable and can bio-accumulate in the natural environment. $SiO_2$ NPs are a class of NPs that has great potential for scientific, biological, and medical research applications [64].

$SiO_2$ NP has been shown to decrease the mRNA expression of *PARP-1* [65], an important DNA repair gene, in human keratinocyte HaCaT. This decrease of expression could be rescued by knockdown of DNMT1 [65]. DNA methylation levels of *PARP-1* promoter has been found increased gradually with the increasing of $SiO_2$ NP concentration, suggesting epigenetic effects play a role in regulating *PARP-1* expression level by $SiO_2$ NP [65]. Nevertheless, it has also been reported that nano and mircro-sized $SiO_2$ decreased global DNA methylation and the related methyltransferase including DNMT1, DNMT3a and MBD2 [66], indicating epigenetic effects of $SiO_2$ NP might be involving writers, readers, and erasers of DNA methylation.

### miRNA induced gene expression change

A large portion of the genome is transcribed into RNA with a significant portion of non-coding RNA (ncRNA) that function as structural, catalytic, or regulatory RNAs, rather than encoding proteins [67]. Although the function of most of the newly identified ncRNAs is yet to be elucidated, emerging evidence has shown that ncRNAs play an important role in chromatin remodeling and epigenetic control of transcription [67]. MicroRNAs (miRNAs) are a group of small ncRNAs that mediate posttranscriptional gene silencing through degradation of mRNA or inhibition of mRNA translation [68]. A complicated feedback network of miRNAs and other epigenetic pathways appears to post-transcriptionally repress gene expression including those signal molecules and thus are critical for many cellular pathways, and to organize the whole gene expression profile [61,69].

Gold NPs (AuNP) has attracted a lot of attention from material scientists for biomedical applications due to their unique features. AuNPs preferentially accumulate at sites of tumor growth/inflammation and their intense photophysical properties facilitate biodiagnostic assays such as HCG pregnancy test [70]. Because of their versatile optical properties, AuNPs have enabled optical imaging of cells with a wide variety of contrast [70].

The epigenetic effects of AuNPs appear most frequently as miRNA levels change [71,72]. Twenty-eight microRNAs were found at significantly altered levels in maternaly exposed fetal lungs, and 5 were up-regulated in fetal liver. Let-7a and miR-183 were significantly up-regulated in both organs [71]. The outcome of the up regulation of these miRNA levels remain elusive, given that Let-7a expression level has been found negatively correlated to lung cancer [73] while miR-183 has been found positively correlated to lung cancer [74]. *In vitro* study using Au Nps exposed human fetal lung fibroblast has shown altered gene expression accompanied with up-regulation of miRNA-155 [72]. A reverse correlation has been established between miRNA-155 levels and *PROS1* expression levels, albeit this has yet to be determined if this is a direct effect [72]. The *PROS1* gene encodes for Protein S, a plasma glycoprotein that is involved in thrombus formation in the pulmonary

vasculature giving rise to adverse outcomes such as lung infraction [75,76].

Different NPs might have similar epigenetic effects in terms of regulating miRNA expression. *In vitro* epigenetic effects of co-regulated miRNAs such as miR-34s, miR-21 and miR-29a were found in $Fe_2O_3$ NPs, cadmium telluride quantum dots (CdTe QDs) and multi wall carbon nanotubes (MW-CNTs) treated cells [77]. Many miRNAs were co-regulated after two out of three nano-material exposure, which suggested the similarity of epigenetic effects of NPs [77]. However, not much overlap was found when comparing regulated miRNA among these three kinds of NPs treated cells with AuNP treated *in vivo* tissues [71,72,77]. This discrepancy might due to different methods that were employed to detect miRNA levels in these studies, which were SOLiD (Sequencing by Oligonucleotide Ligation and Detection) sequencing and miRCURY LNA microRNA Array, respectively [71,77].

### Histone code

Histone tail modifications have emerged as important players in epigenetic regulation of gene expression and other chromatin associated processes. The four core histones, H2A, H2B, H3, and H4; are subject to a methylation, acetylation, phosphorylation, and ubiquitination [78]. These marks are thought to exert their function through direct modulation of chromatin structure and thereby formulating a histone code. The signal is then processed through histone code readers that feature modification-specific binding domains [78].

Quantum dots (QDs) are semiconductor nanocrystals with unique optical properties that are used extensively for *in vitro* observations of cellular mechanisms and for *in vivo* studies aimed at understanding the bio distribution of nanoparticles upon systemic injection [79]. Negatively charged CdTe QDs are capable of rapid nuclear accumulation in cells through phagocytosis [80]. These QDs showed a strong interaction to the core histones and cell nuclear extractions when compared with its interaction with bovine serum albumin (BSA), DNA and RNA [81]. Global hypoacetylation of histones was observed in breast cancer cells in an *in vitro* study. This chromatin condensation is associated with decreased gene transcription and increase in transcription of pro-apoptoticgenes such as *Bax upright Puma* [82].

## Conclusion Remarks

The controversies associated with NP toxicity studies (not limited to) discussed above are calling for a standard genotoxicity testing battery to cover a wide range of mechanisms. Many toxicology studies are lacking data showing NP aggregation and dissolution which occurs in sample media, let alone the changes in bioavailability and toxicity. It has been proposed that for the purpose of genotoxicity analysis of NPs, the OECD standardized methods should be employed; *in vivo* assays should be included to correlate with *in vitro* results; and more rigorous physicochemical characterization of particle-types should be conducted [83]. The interaction of nanoparticles and natural organic matter that occurs during waste processing results in a nanoscale coating of the nano materials, which dramatically changes their surface chemistry, aggregation, deposition, and toxic properties [84]. Engineered nanoparticles in natural systems are subject to a dynamic physical and chemical environment; therefore the toxicity analysis using their "as manufactured" state might not be thorough and comprehensive. More detailed and thoughtful design is needed for accurate risk assessment of NPs.

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