

The Genetic and Epigenetic Toxicity of Silica Nanoparticles: An Updated Review

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Abstract: Silica nanoparticles (SiNPs) are widely used in biomedical fields, such as drug delivery, disease diagnosis, and molecular imaging. An increasing number of consumer products containing SiNPs are being used without supervision, and the toxicity of SiNPs to the human body is becoming a major problem. SiNPs contact the human body in various ways and cause damage to the structure and function of genetic material, potentially leading to carcinogenesis, teratogenicity and infertility. This review summarizes SiNPs-induced genetic and epigenetic toxicity, especially to germ cells, and explore their potential mechanisms. SiNPs cause genetic material damage mainly by inducing oxidative stress. Further, the molecular mechanisms of epigenetic toxicity are discussed in detail for the first time. SiNPs alter DNA methylation, miRNA expression, histone modification and inhibit chromatin remodeling by regulating epigenetic-related enzymes and transcription factors. This review is beneficial for investigating potential solutions to avoid toxicity and provide guidance for better application of SiNPs in the biomedical field.

Keywords: silica nanoparticles, genotoxicity, epigenetic, DNA damage, germ cells

Introduction

Silica nanoparticles (SiNPs) are among the three most commonly used nanomaterials in the world. Owing to their controllable particle size, large specific surface area, and presence of silanol groups, SiNPs exhibit unique physicochemical properties and excellent biocompatibility.¹ In the biomedical field, SiNPs have been introduced into the human body for drug delivery, disease diagnosis and molecular imaging.²⁻⁴ SiNPs can contact the human body through intravenous injection, lung inhalation, skin contact and gastrointestinal routes and have toxic effects on many organs, including the lungs, liver, heart, brain, spleen, and kidneys.⁵⁻¹⁰ As an increasing number of consumer products containing nanomaterials are used without supervision, the toxicity of nanomaterials to the human body is becoming a major problem.

SiNPs are mainly divided into two types: crystalline SiNPs and amorphous SiNPs. Crystalline SiNPs are released from the natural environment and from construction and industrial processes. Amorphous SiNPs are divided into mesoporous SiNPs and nonporous SiNPs on the basis of the presence or absence of pores.³ Amorphous SiNPs are widely used in food, cosmetics, the automotive industry, and construction, among other industries.¹¹ The commonly reported toxicities of SiNPs are respiratory toxicity, immunotoxicity, cardiovascular toxicity, etc.¹²⁻¹⁴ Therefore, it has become necessary to understand the effects and hazards of SiNPs upon exposure to the human body. Recently, the genetic and epigenetic toxicity of SiNPs has attracted widespread attention and are important parts of evaluating SiNPs' safety. Any abnormal changes in gene information can affect gene expression and pose a threat to human health.

The DNA sequence is the basis of inheritance and the skeleton of genes.¹⁵ Genotoxicity refers to genetic material damage at the base, molecular, and chromosome levels, including gene mutations, DNA damage and chromosomal damage.¹⁶ Epigenetics refers to heritable changes in gene expression that occur without altering the DNA structural sequence, including

modifications of DNA or chromatin structures or proteins related to them.¹⁷ Therefore, genetic and epigenetic abnormalities may affect gene expression and induce toxicity. Genetic and epigenetic toxicity can cause harm, such as cancer, teratogenicity, and infertility, by affecting biological genetic information, resulting in serious health consequences. In this review, we summarize the genetic and epigenetic toxicity induced by SiNPs, elucidate the potential adverse effects of such toxicity (Figure 1), and further discuss the potential underlying molecular mechanisms involved. Considering the genetic effects of reproductive system toxicity on offspring, we paid special attention to genetic and epigenetic toxicity in the reproductive system. This review provides guidance for evaluating the safety of SiNPs and preventing their potential adverse effects on the human body so that they can be better applied in the biomedical field in the future.

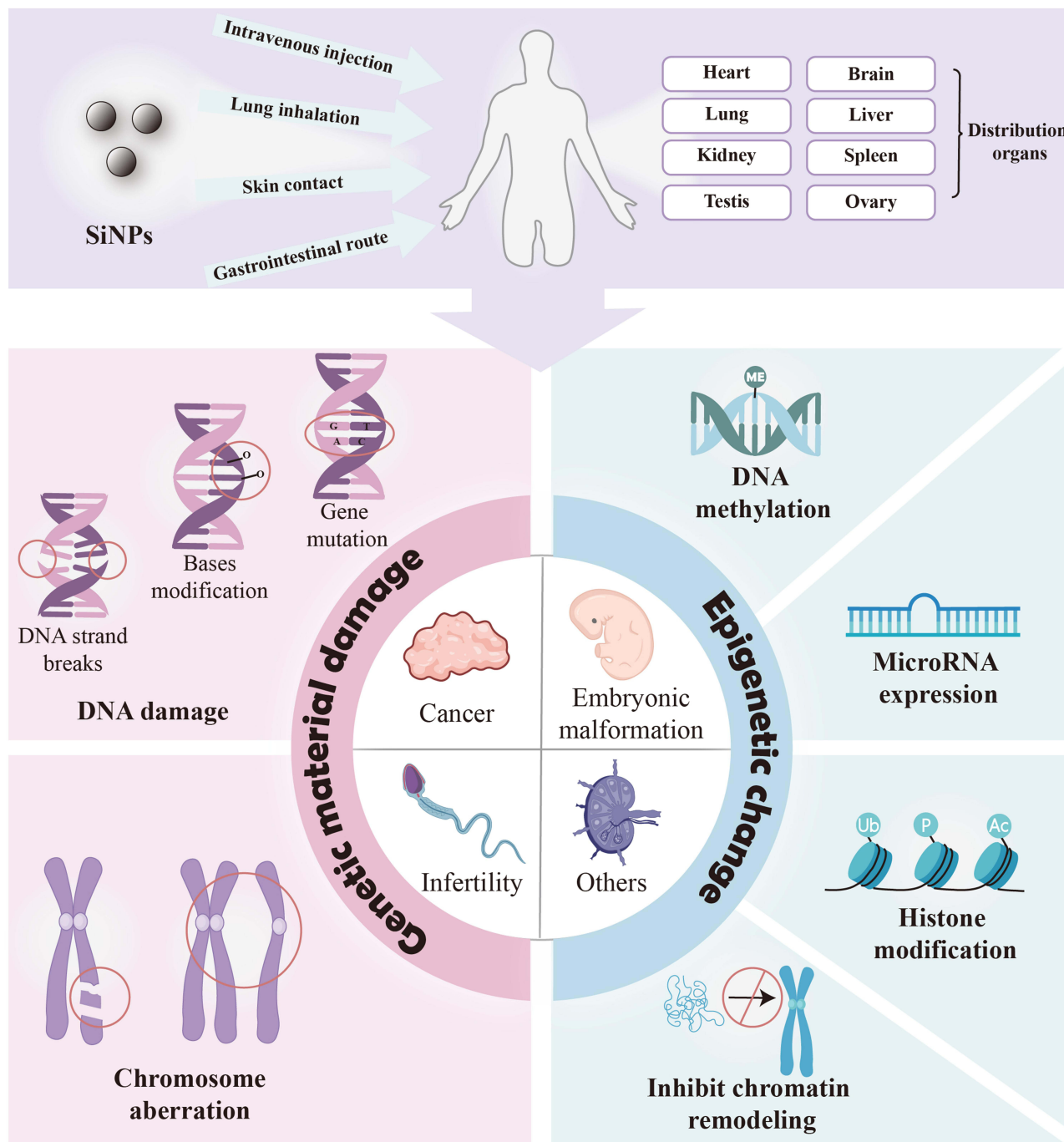


Figure 1 Genetic and epigenetic toxicity and potential risks of silica nanoparticles. SiNPs enter the human body and induce genetic material damage and abnormal epigenetic changes, causing potential risks such as carcinogenesis, teratogenesis and infertility.

Genetic and Epigenetic Toxicity of SiNPs Causes Potential Risks SiNPs Increase the Risk of Cancer Occurrence

The occurrence of cancer is closely related to genetic mutations. Gene mutations affect the steady-state development of key cell functions, causing uncontrolled cell growth and driving the occurrence of cancer.¹⁸ These cancer-driving genes include oncogenes and tumor suppressor genes, which are affected by point mutations, translocations or copy number changes.¹⁹ Previous studies have shown that NPs are harmful components of pollution particles in the air.²⁰ Inhaling tungsten carbide cobalt NPs can lead to “hard metal lung disease”, doubling the risk of lung cancer.²¹ Long-term exposure to inhalable crystalline silica can induce silicosis and may even lead to lung tumors.²² Amorphous SiNPs increase the frequency of gene mutations and induce the malignant transformation of some somatic cells, leading to the loss of growth inhibition signals or cell dysfunction.^{23–25} Therefore, the genotoxicity of SiNPs causes precancerous transformation and increases the risk of cancer. However, whether genetic material damage caused by SiNPs leads to cancer is related to the dose, exposure time, cell sensitivity, etc.

SiNPs Cause Embryonic Malformation and Behavioral Changes

Embryonic development is tightly regulated by genes, and DNA damage is considered the main mechanism leading to abnormalities in embryonic development.²⁶ SiNPs may penetrate the placental barrier, accumulate in fetal tissue, induce DNA damage, and lead to embryonic developmental abnormalities, including pericardial and yolk sac edema, blood clots and delays in embryonic development.^{27,28}

A low concentration of SiNPs does not cause changes in the developmental morphology of zebrafish embryos, but it causes behavioral changes in embryonic light movement and the larval motor response.²⁹ However, SiNPs can enrich pollutants. When absorbing environmental pollutants such as cadmium and tetrabromobisphenol A, SiNPs amplify the teratogenic toxicity of these pollutants and cause abnormal embryonic development.^{30–32} In addition, large SiNPs are blocked by placental chorionic villi and do not cause embryonic development abnormalities, whereas small SiNPs, such as those that are ≤ 70 nm, can penetrate the placental barrier and accumulate in the placenta, fetal liver, and brain, causing malformations of the embryo.³³ Therefore, exposure to small SiNPs should be avoided during pregnancy.

SiNPs Increase the Risk of Infertility

DNA damage and histone modification abnormalities in germ cells induced by SiNPs can lead to infertility.^{34,35} Many studies have reported the negative impact of SiNPs on fertility.^{36,37} In the male reproductive system, SiNPs can penetrate the blood–testis barrier and accumulate in the testes and epididymis, leading to abnormal sperm morphology, decreased sperm count, and spermatogenesis dysfunction.^{34,36} In the female reproductive system, SiNPs are internalized and accumulate in the ovaries, leading to granulosa cell apoptosis and follicular atresia, impairing the survival and developmental ability of ovarian cells.^{32,37} One study revealed that damage caused by Stöber SiNPs to the reproductive system could be reversed after exposure was stopped, suggesting that the adverse effects of SiNPs may be temporary.³⁵

Other Diseases

The genotoxicity induced by SiNPs may also promote the occurrence of autoimmune diseases, endocrine diseases, etc. For example, SiNPs damage macrophage DNA and chromosomes, induce immune system toxicity, and may cause immune system diseases.¹³ DNA hypermethylation leads to abnormal insulin secretion and promotes the occurrence of type 2 diabetes mellitus.³⁸

The Genetic and Epigenetic Toxicity of SiNPs Genetic Material Damage

SiNPs cause genetic material damage, which has been reported to occur mainly in human lung epithelial cells, fibroblasts, and germ cells.^{35,39} SiNPs can cause damage to genetic material at the base, DNA molecular and chromosomal levels, leading to genotoxicity. DNA damage induced by SiNPs includes gene mutation, oxidative modification of bases, or DNA strand breaks.^{40–44} For example, exposure to spherical amorphous-SiNPs causes gene mutation and

comet-like nuclear changes (Figure 2A and D).^{41,45} Comet-like nuclear changes are caused by DNA breakage and migration away from the nucleus. In addition, SiNPs induce the phosphorylation of histone family member X (γ -H2AX), which is a specific biomarker of DNA double-strand breaks (Figure 2B and C).⁴³ The type of DNA damage is influenced by the size of the NPs. Smaller NPs are prone to insertion into DNA molecules, causing base mismatch, whereas larger NPs tend to bind to the DNA strand, causing DNA strand breakage.^{46,47}

In addition, DNA damage caused by NPs also includes abasic sites and DNA adducts. Graphite oxide induces abasic sites in DNA, ie, sites with neither a purine base nor a pyrimidine base.⁴⁹ NPs with electrophilic groups, such as magnetic nanoparticles, combine covalently with DNA to form DNA adducts.⁵⁰ However, whether SiNPs induce abasic sites and DNA adducts is unclear.

At the chromosome level, SiNPs induce chromosomal aberrations, including structural and numerical aberrations.⁵¹ Amorphous SiNPs of four sizes (100 nm, 50 nm, 25 nm, and 10 nm) induce dose-dependent micronucleus frequency changes (Figure 2E).⁴⁵ SiNPs may also cause chromosomal numerical aberration by inhibiting chromosome segregation or inducing whole chromosome loss in the cell division stage.^{52–54} In conclusion, SiNPs induce DNA and chromosomal damage, posing potential risks to the human body, such as cancer, deformities, and infertility.

Abnormal Epigenetic Changes

Changes in DNA Methylation

SiNPs can induce abnormal DNA methylation, regulate gene expression and cause toxicity. DNA methylation is currently the most widely studied epigenetic mechanism.⁵⁵ DNA methylation inhibits gene expression by altering transcription factor binding and chromatin conformation.⁵⁶ It regulates many cellular processes, including cell differentiation, embryonic development, and gene transcription.⁵⁷ Abnormal (high or low) methylation affects genomic stability and has many adverse consequences. Hypermethylation of specific promoter regions and global DNA hypomethylation are closely related to the occurrence of cancer. In addition, abnormal methylation can lead to autoimmune diseases, respiratory diseases, skin diseases, etc.³⁸

SiNPs induce extensive DNA methylation changes in GC-2 cells, including 51.0% hyperdifferentially methylated regions and 49.0% hypodifferentially methylated regions.⁵⁸ This abnormal DNA methylation leads to abnormal transcription and translation, mitochondrial damage, and sperm apoptosis. SiNPs also induce abnormal DNA methylation changes in specific genes. SiNPs inhibit outer dense fiber 1 (Odf1) and Bcl-x1 in spermatocytes by inducing Crem hypermethylation, leading to sperm bundle structure and spermatocyte apoptosis, thereby disrupting spermatogenesis.⁵⁹ Notably, abnormal DNA methylation induced by SiNPs can affect offspring. Prenatal exposure to high-dose food-grade SiNPs E 551 can lead to hypermethylation in the maternal and fetal liver, causing liver metabolic disorders and teratogenicity (Figure 3).⁶⁰ Long interspersed element-1 (LINE-1) and Alu elements are important noncoding elements in the human genome, and changes in their methylation levels are closely related to the development of cancer and autoimmune diseases. SiNPs have been shown to induce DNA hypomethylation of Alu elements in HaCaT cells but have no effect on the DNA methylation levels of LINE-1.⁶¹ These findings suggest that the impact of SiNPs on DNA methylation is DNA sequence specific. However, the current research on the impact of SiNPs on DNA methylation is mostly based on in vitro experimental models, and further verification is needed to determine whether abnormal methylation occurs in vivo and causes corresponding adverse consequences.

Changes in microRNA Expression

MicroRNAs (miRNAs) can affect gene expression by binding to the 3' untranslated region (3'-UTR) of mRNAs to increase the degradation of posttranscriptional mRNAs or inhibit gene translation.⁶² miRNAs play important roles in cell differentiation, proliferation, apoptosis, and angiogenesis. SiNPs induce abnormal expression of miRNAs. The abnormal expression of these miRNAs leads to reproductive dysfunction, endothelial dysfunction, and even tumorigenesis.^{25,63–66}

SiNPs induce abnormal miRNA expression in germ cells. These abnormal changes in miRNA expression interfere with DNA replication, DNA repair, fatty acid metabolism, and autophagy, resulting in damage to reproductive system function. SiNPs increase the expression of 10 types of miRNAs and decrease the expression of 5 types of miRNAs, which are functionally enriched in DNA replication and fatty acid metabolism, in GC-2 spd cells (Figure 4). Among

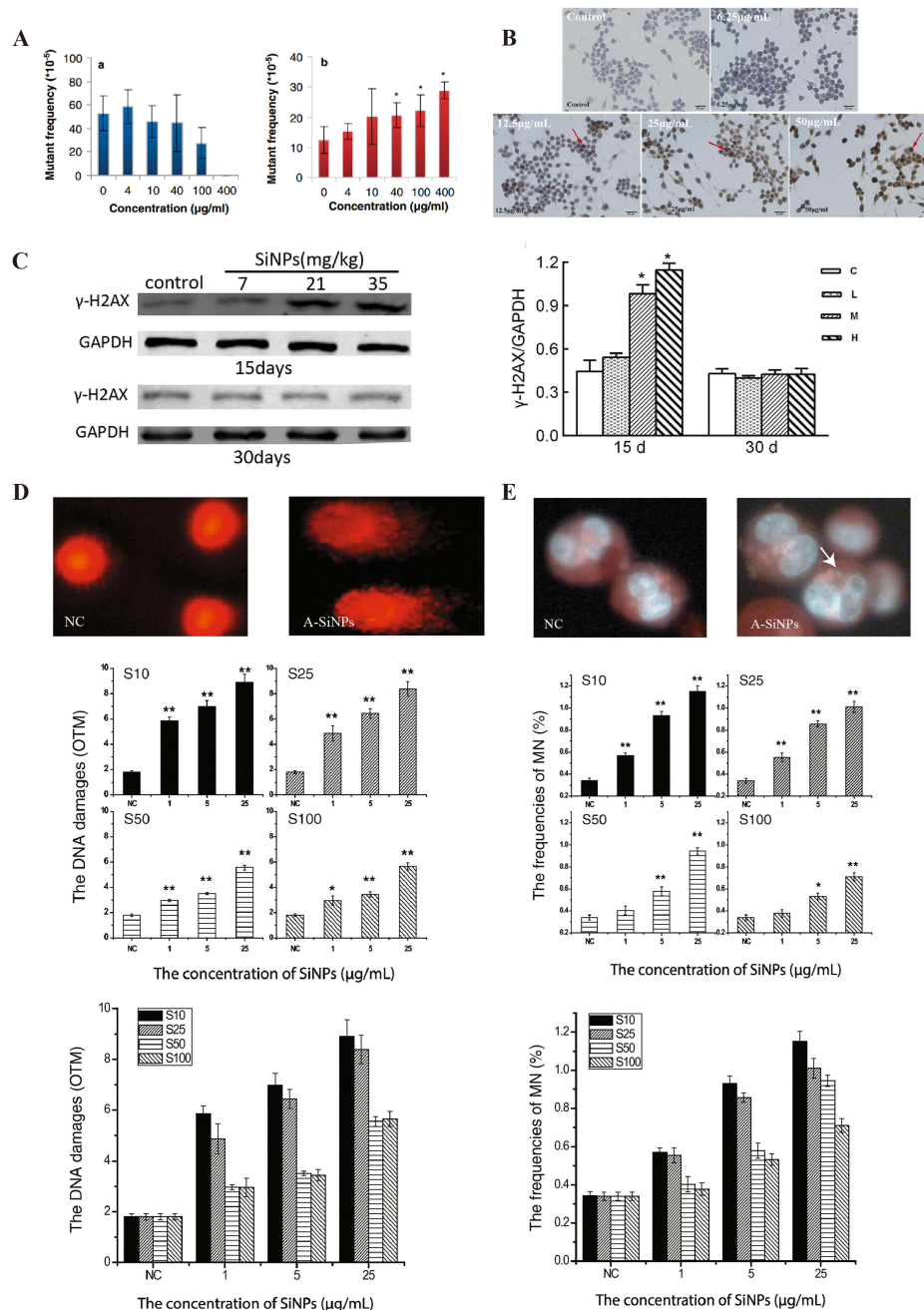


Figure 2 Genetic material damage induced by SiNPs. **(A)** Mutant frequencies in MEF-LacZ cells exposed to (a) 10-nm and (b) 30-nm spherical A-SiNPs. The 30-nm SiNPs increased the mutation frequency in a dose-dependent manner. $^*P < 0.05$. Source: Park MVDZ, Verharen HW, Zwart E, et al. Genotoxicity evaluation of amorphous silica nanoparticles of different sizes using the micronucleus and the plasmid lacZ gene mutation assay. *Nanotoxicology*. 2011;5(2):168–181. reprinted by permission of the publisher (Taylor&FrancisLtd, <http://www.tandfonline.com>).⁴¹ **(B)** Effect of SiNPs on the expression of γ -H2AX in GC-2 cells after 24 h of exposure. γ -H2AX (marked with red arrows) is a biomarker of DNA strand breaks. Obvious γ -H2AX-positive staining was observed in the 25 and 50 $\mu\text{g/ml}$ SiNPs groups. Source: Reprinted from Zhang J, Liu J, Ren L, et al. Silica nanoparticles induce abnormal mitosis and apoptosis via PKC-delta mediated negative signaling pathway in GC-2 cells of mice. *Chemosphere*. 2018;208:942–950. With permission from Elsevier.⁴³ **(C)** Western blot analyses of γ -H2AX proteins in the ovaries of Balb/c female mice after exposure to SiNPs by intratracheal instillation. On the 15th day after the first dose, the expression of γ -H2AX significantly increased. On the 30th day after the first dose, the γ -H2AX level did not obviously differ among the groups. C, control group; L, 7 mg/kg SiNPs group; M, 21 mg/kg SiNPs group; H, 35 mg/kg SiNPs group. $^*P < 0.05$. Source: Reprinted with permission from Liu J, Yang M, Jing L, et al. Silica nanoparticle exposure inducing granulosa cell apoptosis and follicular atresia in female Balb/c mice. *Environ Sci Pollut Res Int*. 2018;25(4):3423–3434. Springer Nature.⁴⁸ **(D)** Changes in the nuclear shape of HUVECs, as determined by the alkaline comet assay. The typical “comet” shapes of the cell nuclei were observed in the A-SiNPs group (exposed for 4 h). DNA damage was interpreted by the OTM. The OTM values revealed the dose- and size-dependent effects on DNA damage induced by SiNPs. $^*P < 0.05$, $^{**}P < 0.01$. Source: Reprinted with permission from Zhou F, Liao F, Chen L, Liu Y, Wang W, Feng S. The size-dependent genotoxicity and oxidative stress of silica nanoparticles on endothelial cells. *Environ Sci Pollut Res Int*. 2019;26(2):1911–1920. Springer Nature.⁴⁵ **(E)** Chromosomal damage in HUVECs was detected via a cytokinesis-block MN assay. MNs in binucleated cells (marked with white arrows) exposed to A-SiNPs. A-SiNPs (exposed for 24 h) had dose-dependent effects on MN %. The results are presented as the percentage of micronucleated cells (MN%) per 1000 binucleated cells. $^*P < 0.05$, $^{**}P < 0.01$. Source: Reprinted with permission from Zhou F, Liao F, Chen L, Liu Y, Wang W, Feng S. The size-dependent genotoxicity and oxidative stress of silica nanoparticles on endothelial cells. *Environ Sci Pollut Res Int*. 2019;26(2):1911–1920. Springer Nature.⁴⁵ MEF-LacZ, containing lacZ as a reporter gene; A-SiNPs, amorphous silica nanoparticles; γ -H2AX, histone family member X phosphorylation; OTM, olive tail moment; GC-2 cells, spermatocyte lines; MN, micronucleus.

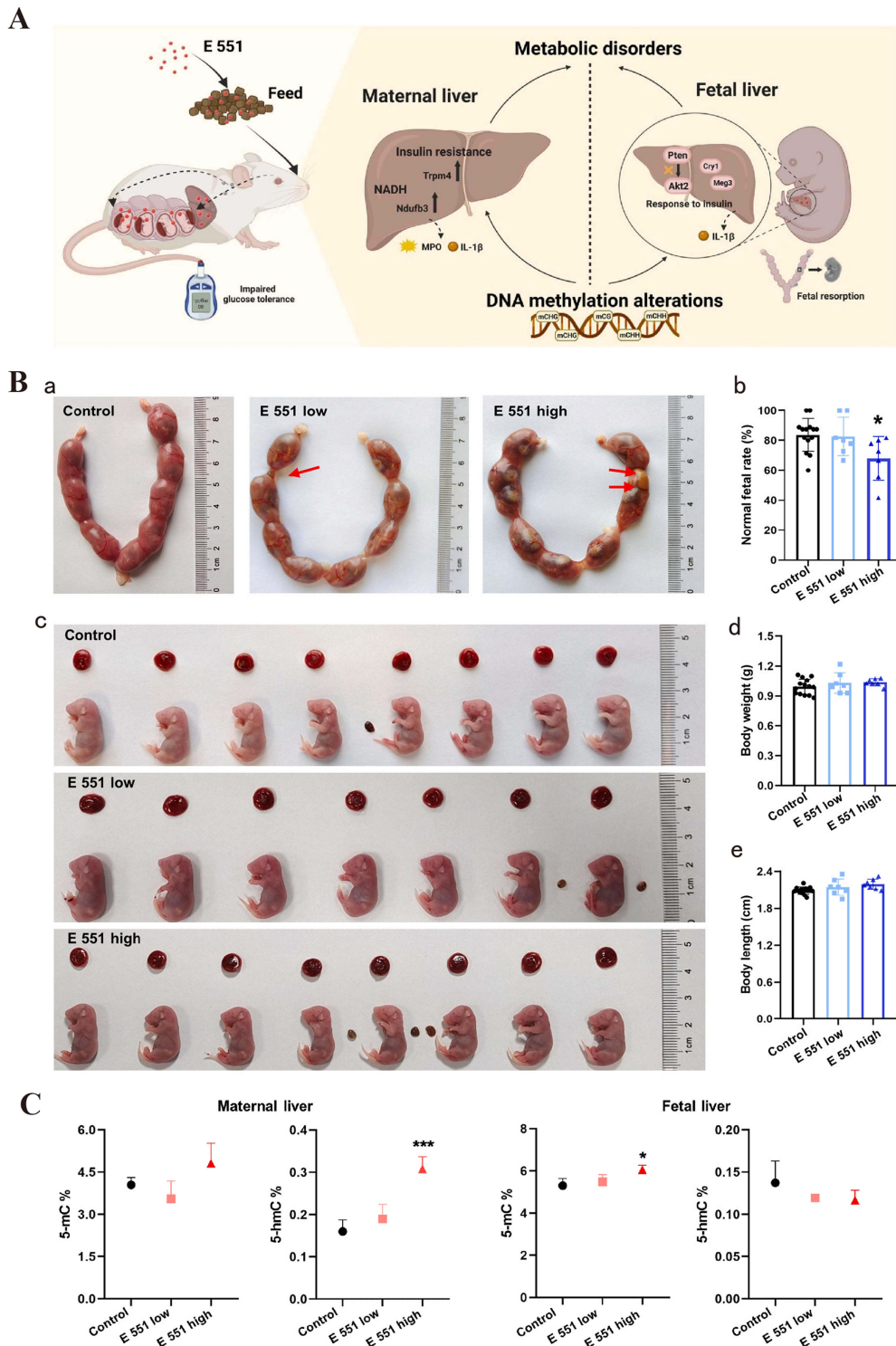


Figure 3 Food-grade SiNPs (E 551) induce genome-wide DNA methylation changes in mothers and fetuses. **(A)** E 551 accumulated in maternal and fetal liver tissues, causing genome-wide DNA methylation changes in liver tissues after high-dose prenatal exposure. The methylation and altered expression of genes are related mainly to glycolipid metabolism, which impairs glucose tolerance in pregnant mice. E 551 has a risk of inducing metabolic disorders in both the maternal and fetal liver, leading to fetal resorption. **(B)** (a) Representative uterine morphology after E 551 exposure at GD19. The red arrows indicate that low-dose and high-dose E 551 resulted in fetal resorption. (b) Normal fetal rates after E 551 exposure. High-dose exposure decreased normal fetal rates. (c) Representative fetal and placental morphology at GD19. (d and e) Weights (d) and body lengths (e) of all fetuses in the control group (n = 14), low-dose E 551 group (n = 7), and high-dose E 551 group (n = 7). **(C)** Genomic 5-mC and 5-hmC levels in the maternal liver and fetal liver; n=7 for each group. 5-mC levels increased in high-dose E 551-exposed livers. The 5-hmC levels in the maternal liver increased only in the high-dose E 551 group. * $P < 0.05$, *** $P < 0.001$, compared with the control group. Source: Reprinted from Zhan Y, Lou H, Shou R, et al. Maternal exposure to E 551 during pregnancy leads to genome-wide DNA methylation changes and metabolic disorders in the livers of pregnant mice and their fetuses. *J Hazard Mater.* 2024;465:133233. Copyright 2024, with permissions from Elsevier.⁶⁰ GD19, gestational day 19; 5-methylcytosine; 5-hmC, 5-hydroxymethylcytosine.

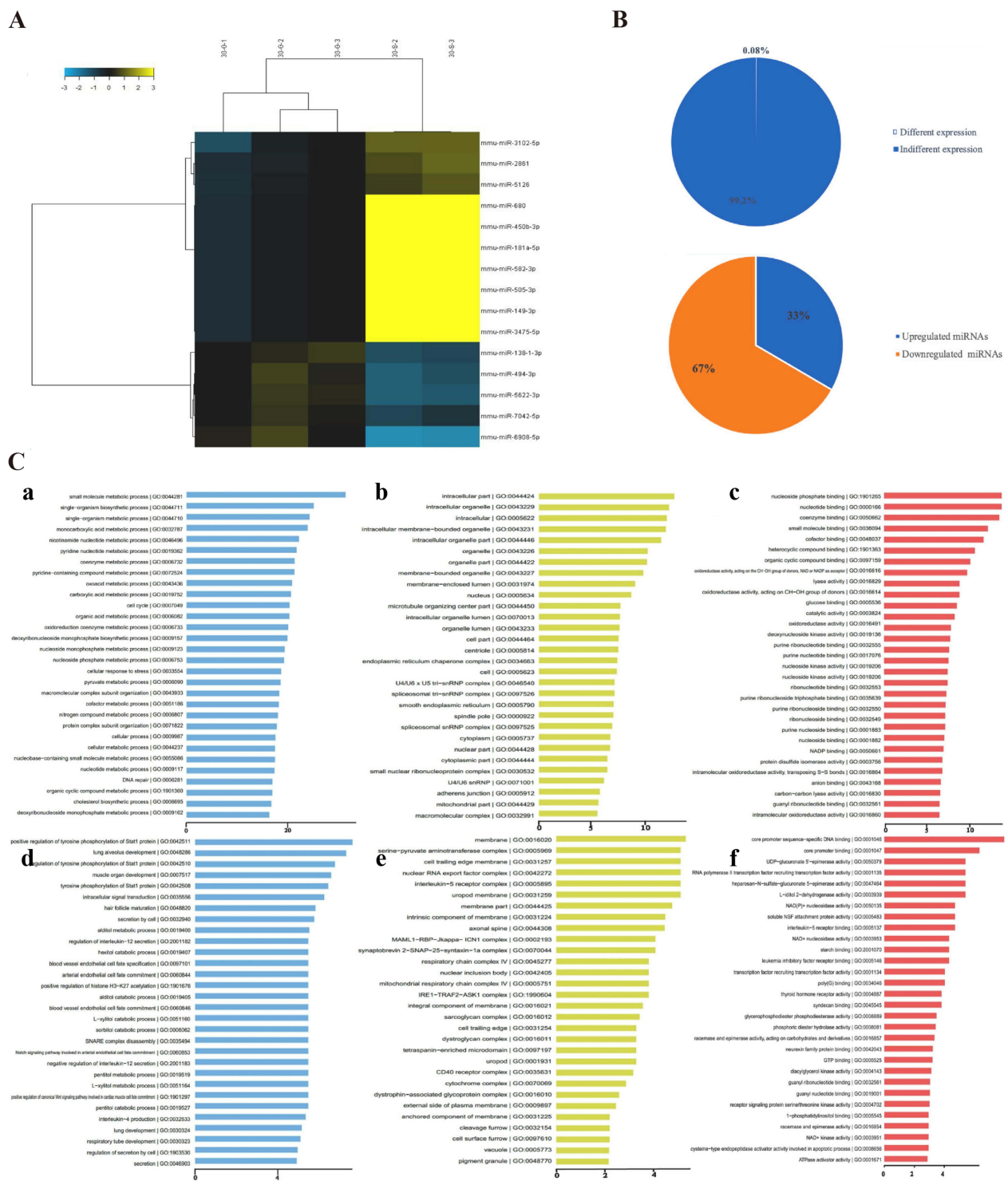


Figure 4 SiNPs-induced changes in the miRNA expression profile of GC-2 spd cells. **(A)** The expression levels of 15 miRNAs changed in GC-2 spd cells after SiNPs (5 µg/mL, 24 h) exposure. The relative up- and downregulation of miRNAs are indicated by yellow and blue, respectively. **(B)** Percentages of differentially expressed miRNAs in GC-2 spd cells. Fifteen miRNAs (0.08%) were differentially expressed. Among them, 5 were upregulated (33.3%), and 10 were downregulated (66.7%). **(C)** Pathways associated with significantly up- and downregulated miRNAs according to the GO enrichment database. Top 30 significant GO terms for the 15 miRNAs. (a) Biological processes of upregulated miRNA target genes. Biological processes primarily involve small-molecule metabolic processes. (b) Cellular components of upregulated miRNA target genes. Cellular components primarily include intracellular components. (c) Molecular functions of upregulated miRNA target genes, which involve mainly nucleoside phosphate binding. (d) Biological processes of downregulated miRNA target genes, which primarily involve the regulation of tyrosine phosphorylation of the Stat1 protein. (e) Cellular components of downregulated miRNA target genes, which primarily involve the membrane. (f) Molecular functions of downregulated miRNA target genes, which involve mainly core promoter sequence-specific DNA binding. X-axis, negative logarithm of the P value (-LgP); the larger the number is, the smaller the P value. *Source:* Reprinted from Zhou G, Ren L, Yin H, et al. The alterations of miRNA and mRNA expression profile and their integration analysis induced by silica nanoparticles in spermatocyte cells. *NanoImpact.* 2021;23:100348. Copyright 2021, with permission from Elsevier.⁶⁷ GC-2 spd cells, spermatocyte lines.

them, miRNA-450b-3p inhibits cell cycle progression, and miRNA-138-1-3p may inhibit fatty acid metabolism.⁶⁷ Zhao et al reported that SiNPs inhibit the expression of the DNA repair-related protein zinc finger CW-type and PWWP domain containing 1 (ZCWPW1) by increasing the expression of miRNA-5622-3p, leading to DNA repair failure in GC-2 spd cells.⁶⁵ SiNPs have also been shown to upregulate miRNA-494, which ultimately causes autophagy dysfunction and spermatocyte death.⁶⁸ Moreover, another study revealed that SiNPs downregulated miRNA-450b-3p expression to increase cytoskeletal protein expression, thus disrupting the mitochondrial structure and inducing cell apoptosis, ultimately inhibiting sperm development.⁶⁴ Notably, the reproductive dysfunction caused by miRNA deregulation is caused by short-term exposure (less than 3 months) to SiNPs, and long-term effects have not yet been reported. This may be because long-term tracking and observation require a significant amount of time and manpower.

SiNPs activate the IL6R/STAT/TF signaling pathway by downregulating miRNA-451a, leading to endothelial dysfunction and thrombosis.⁶⁶ In addition to causing dysfunction, abnormal miRNA expression may lead to severe consequences of tumorigenesis. Amorphous SiNPs affect ATP5H/SOD1 and EIF4G2/PAPPC1 gene expression through the upregulation of miRNA-3648/572/661 and downregulation of miRNA-4521, thereby promoting the occurrence of cancer.²⁵ These findings suggest that these four miRNAs may be potential biomarkers of cancer and can serve as potential therapeutic targets for preventing the toxicity of SiNPs. However, the above studies are based on microarray data predictions and require wet experiments to demonstrate the interaction between identified genes and miRNA expression, as well as their impact on cell proliferation and apoptosis.

Changes in Histone Modifications

Chromosomes are composed of DNA and histones, which bind together. Histones are classified into five types: H1, H2A, H2B, H3, and H4. The N-terminal and C-terminal tails of histones can undergo posttranslational modifications, including acetylation, ubiquitination, phosphorylation, and methylation. The different histone modification methods determine the structural state of chromatin condensation or loosening and play a role in transcriptional regulation, replication, repair, and recombination.⁵⁶ The abnormal modification of histones can lead to abnormal gene expression, which is closely related to the pathogenesis of cancer, neurodevelopmental disorders, and autoimmune diseases.³⁸

SiNPs can induce abnormal states of ubiquitination, phosphorylation and acetylation of histones. SiNPs inhibit the ubiquitination of H2A and H2B in the sperm nucleus, leading to sperm production disorders (Figure 5A and B).^{35,69} Histone phosphorylation is a key phenomenon in DNA damage and response at different stages of the cell cycle. SiNPs increase the phosphorylation of H2AX, causing the apoptosis of spermatocytes.³⁵ In addition, SiNPs increase the acetylation levels of histones H3K9 and H3K56, leading to human lung epithelial cell apoptosis.⁷⁰ Histone methylation is the most stable form of modification. It is involved in the activation and inhibition of transcription as well as in the compaction of chromatin. Nanoparticles, such as selenium nanoparticles, can induce abnormal histone methylation, which increases the methylation of histones H3K9 and H3K27.⁷¹ However, the impact of SiNPs on histone methylation has not yet been reported. This requires further exploration.

Inhibiting Chromatin Remodeling

Chromatin remodeling refers to the dynamic process of changing chromatin between concentrated and relaxed states and plays an important role in optimizing cell adaptation and body development.⁷² Chromatin remodeling regulates the accessibility of chromatin to transcription factors.⁷³ This process is achieved through changes in the position and structure of nucleosomes. When remodeling is inhibited, it leads to abnormal gene expression and causes genotoxicity.

Chromatin remodeling occurs during spermatogenesis, in which more than 90% of the core histones in the nucleosome are successively replaced by testicle-specific histone variants, transition proteins (TNPs), and protamine (PRM).⁷⁴ Studies have indicated that nanoparticles may inhibit chromatin remodeling. Titanium NPs inhibit chromatin remodeling by reducing the expression of PRM1 and TNP2 in male mice.⁷⁵ Gold NPs inhibit chromatin remodeling, resulting in increased chromatin instability.⁷⁶

SiNPs inhibit chromatin remodeling in the haploid phase of sperm cells, hindering sperm cell differentiation and ultimately leading to a decrease in sperm quantity and quality (Figure 5C and D).⁶⁹ Notably, after a 15-day withdrawal period, this damage disappeared, indicating that the sperm toxicity induced by SiNPs may be reversible. Whether this

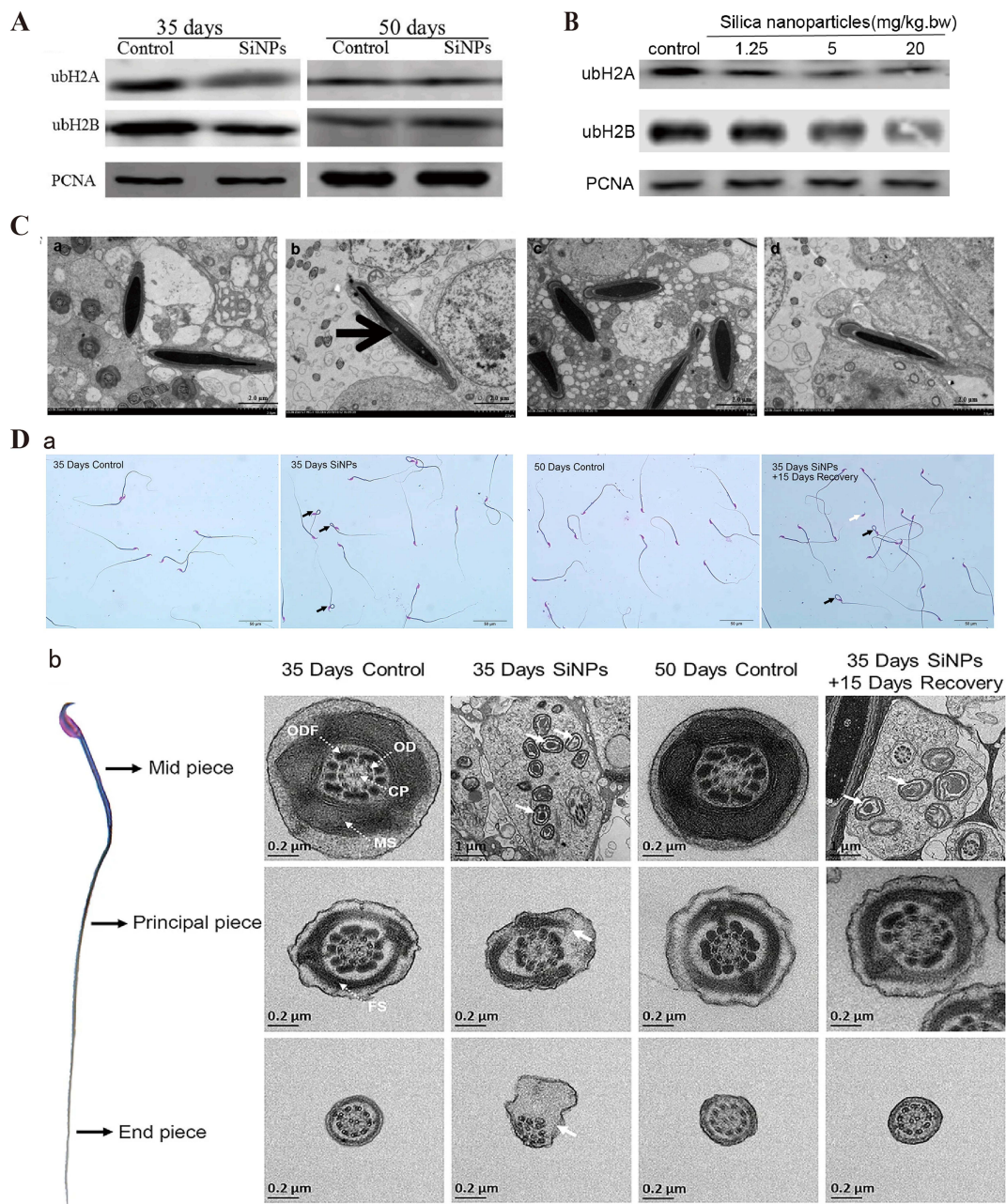


Figure 5 SiNPs inhibit histone ubiquitination and chromatin remodeling. **(A)** SiNPs (20 mg/kg.bw) inhibited ubH2A/ubH2B protein expression in nuclear extracts of elongating spermatids from male ICR mice after intratracheal instillation for 35 days. After the 15-day withdrawal period, the ubH2A/ubH2B levels recovered. *Source:* Reprinted from Liu J, Li X, Zhou G, et al. Silica nanoparticles inhibiting the differentiation of round spermatid and chromatin remodeling of haploid period via MIWI in mice. *Environ Pollut.* 2021;284:117446. Copyright 2021, with permission from Elsevier.⁶⁹ **(B)** Western blot analyses of ubH2A/ubH2B in the nuclear extracts of germ cells from male ICR mice after exposure to SiNPs by intratracheal instillation every 3 days for 15 days. SiNPs inhibited the expression of ubH2A/ubH2B in a dose-dependent manner. The internal control protein was PCNA. *Source:* Reprinted from Liu J, Li X, Zhou G, et al. Silica nanoparticles induce spermatogenesis disorders via L3MBTL2-DNA damage-p53 apoptosis and RNF8-ubH2A/ubH2B pathway in mice. *Environ Pollut.* 2020;265(Pt A):114974. Copyright 2020, with permission from Elsevier.³⁵ **(C)** TEM images showing a defect in DNA condensation in sperm heads after exposure to SiNPs (20 mg/kg.bw) for 35 days. After the 15-day withdrawal period, there was no significant difference in the amount of sperm nuclear chromatin. (a) Control group after 35 days. (b) SiNPs group after 35 days. (c) Control group after the 15-day withdrawal period. (d) SiNPs group after the 15-day withdrawal period. The black thick arrow indicates the less condensed chromatin in the sperm. *Source:* Reprinted from Liu J, Li X, Zhou G, et al. Silica nanoparticles inhibiting the differentiation of round spermatid and chromatin remodeling of haploid period via MIWI in mice. *Environ Pollut.* 2021;284:117446. Copyright 2021, with permission from Elsevier.⁶⁹ **(D)** Effects of SiNPs on sperm quality. (a) Epididymal sperm morphology detected via sperm smears. The black arrows point to sperm folded at the neck, and the white arrow points to sperm with its head falling off. More sperm with abnormal morphology, including neck folding and head shedding, were observed in the 35 days SiNPs group than in the 35 days SiNPs +15 days recovery group. (b) Electron microscope image of the structure of each segment of the sperm flagella. The cross section of the middle piece includes the CP, OD, ODF and MS. The cross section of the principal piece contains the CP, OD, ODFs, and FS. The solid arrows represent abnormal structures. The sperm flagella were significantly damaged in the 35 days SiNPs group. *Source:* Reprinted with permission from Sang Y, Liu J, Dong X, et al. Silica nanoparticles induce male reproductive toxicity via Crem hypermethylation mediated spermatocyte apoptosis and sperm flagella damage. *Environ Sci Pollut Res.* 2024;31(9):13856–13866. Springer Nature.⁵⁹ ubH2A, ubiquitinated H2A; ubH2B, ubiquitinated H2B; PCNA, proliferating cell nuclear antigen; bw, body weight; ICR mice, Institute of Cancer Research mice; CP, central pair; OD, outer doublet microtubules; ODF, outer dense fiber; MS, mitochondrial sheath; FS, fibrous sheath.

reversible damage is cell-specific or environment-specific and when irreversible damage can be caused are unknown. SiNPs affect the maturation of oocytes and interfere with the meiotic division of ova.³² However, it is not yet clear whether the interference of SiNPs in ovum formation is caused by chromatin remodeling.

Genetic and Epigenetic Toxicity Mechanisms of SiNPs

As mentioned above, the genotoxicity of SiNPs mainly manifests as damage to genetic material and abnormal genetic information caused by abnormal epigenetic changes, affecting body development and causing related diseases. The possible molecular mechanisms are discussed below.

Genetic Material Damage

The oxidative stress damage caused by reactive oxygen species (ROS) in genetic materials is the main mechanism of SiNP genotoxicity.^{45,77} ROS may attack bases, deoxyribose or DNA main chains, resulting in nonbulky (nonhelix distortion) and bulky (helix distortion) damage.^{78,79} ROS may also attack chromosomes, causing chromosome breakage and fragmentation.

Mitochondrial dysfunction is one of the mechanisms of SiNP-induced oxidative stress damage in genetic material. On the one hand, owing to the silicon-bonded hydroxyl groups on the particle surface and the unsaturated bonds, SiNPs have a high oxidation capacity, which can induce the electron respiratory transmission chain on the inner membrane of mitochondria to produce ROS (Figure 6A).^{80,81} On the other hand, exposure to SiNPs may lead to the depletion of intracellular antioxidant defense systems, including antioxidant enzymes and reduced glutathione (GSH), resulting in the accumulation of ROS (Figure 6B).^{66,82} In vivo studies have shown that SiNPs reduce the activities of superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and induce excessive ROS production.⁸³ The excessive production of ROS results in mitochondrial swelling and crista rupture (Figure 6C).⁸⁴ Moreover, ROS also damage mitochondrial DNA, leading to a reduction in the mitochondrial membrane potential (MMP) and mitochondrial dysfunction.⁸⁵ These changes cause defects in the transport of mitochondrial iron sulfur cluster proteins and the accumulation of Fe²⁺ in mitochondria, further aggravating oxidative stress damage.⁸⁶ Therefore, a vicious cycle caused by SiNPs occurs between mitochondrial dysfunction and mitochondrial oxidative stress, further promoting the accumulation of ROS in cells and damaging genetic material.

In addition to directly affecting mitochondrial function, amorphous SiNPs promote macrophage or neutrophil aggregation through inflammation and produce reactive oxygen species (ROS) and reactive nitrogen species (RNS) by upregulating NADPH oxidase and nitric oxide synthase (NOS), which cause genetic material damage to cells at the site of inflammation (Figure 7).^{5,87}

Regulatory Mechanism of DNA Methylation

DNA methylation refers to the transfer of a methyl group from S-adenosylmethionine to the fifth carbon of a cytosine residue to form 5-methylcytosine (5-meC) under the catalysis of DNA methyltransferases (DNMTs).⁵⁵ Multiple mechanisms are involved in DNA methylation induced by SiNPs, including the regulation of DNMTs, ten-eleven translocation (TET) and DNA methylation substrates (Figure 8).

In mammals, there are three types of DNMTs: DNMT1, DNMT3a, and DNMT3b. DNMT1 is a DNA methylation maintenance factor that maintains DNA methylation patterns during replication. DNMT3a and DNMT3b are de novo synthesized DNMTs that transfer methyl groups to DNA. Generally, hypermethylation is related to the overexpression of DNMTs, whereas hypomethylation is related to a lack of DNMTs.⁸⁸ SiNPs induce whole-genome hypomethylation by decreasing the expression of DNMT1 and DNMT3a in HaCaT cells (Figure 9A).⁸⁹ Further investigation revealed that knockdown of DNMT1 restored the normal expression and promoter methylation levels of PARP-1, indicating that hypermethylation of the PARP-1 promoter induced by SiNPs is mediated by DNMT1.⁹⁰ The food-grade SiNPs E 551 slightly increased DNMT3a expression, leading to hypermethylation, which was subsequently inherited by the offspring.⁶⁰ SiNPs may promote the transcription of DNMT3a and DNMT3b by activating the JAK2/STAT3 or Akt/Src/STAT3 pathway, which can induce hypermethylation.^{91–95} SiNPs decrease DNMT1 and DNMT3a expression by activating JNK or PKC- δ to phosphorylate p53 or cause the cytoplasmic localization of p53, leading to hypomethylation.^{43,96–98}

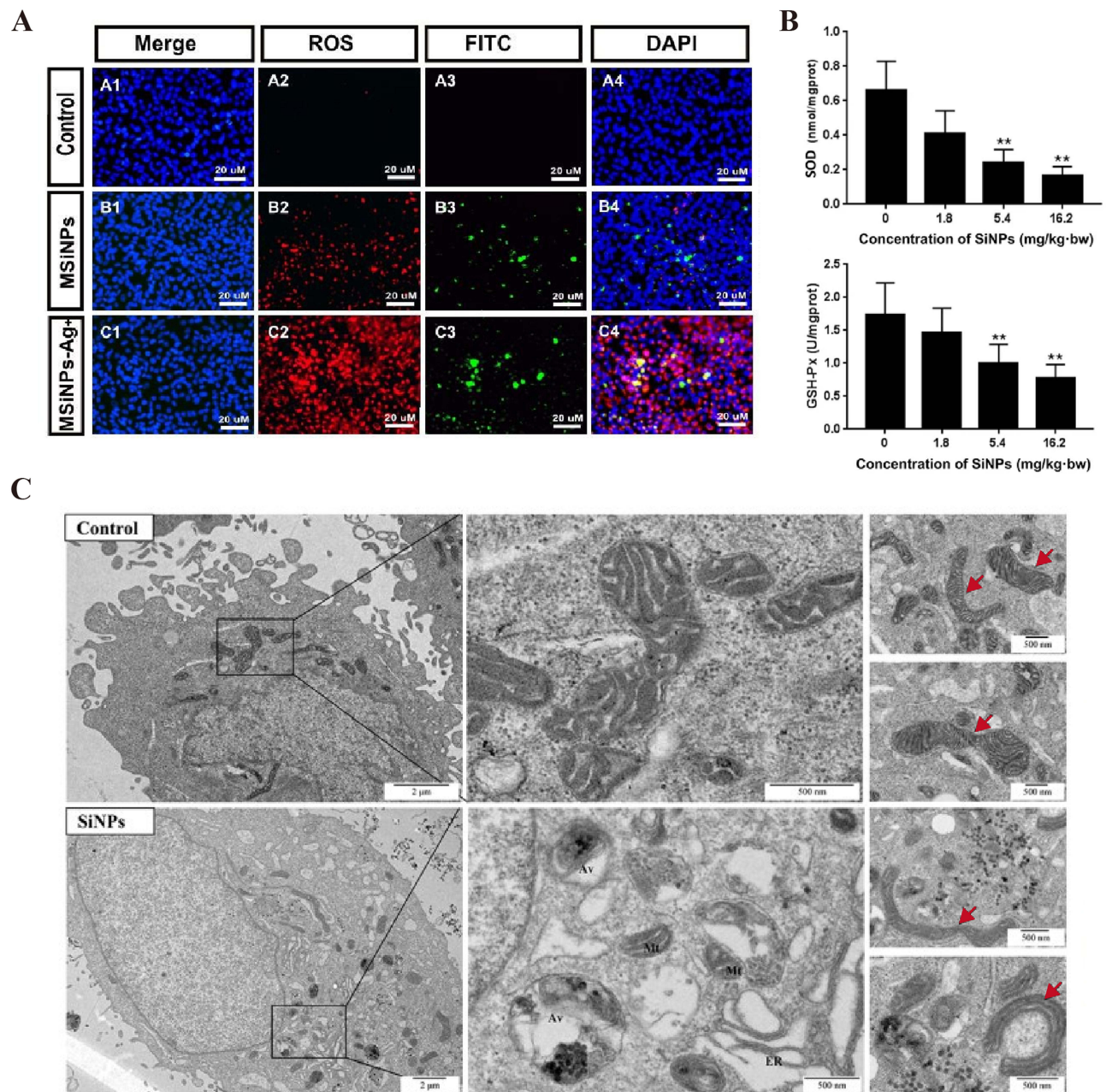


Figure 6 SiNPs induce ROS production and disrupt mitochondrial structure. **(A)** ROS assay of hCECs exposed to 100 $\mu\text{g/mL}$ MSiNPs or MSiNP-Ag⁺ for 24 h. Immunofluorescence images of specific markers in hCECs, including intracellular ROS (red), DAPI (blue) and FITC (green), are shown. The ROS levels of hCECs increased in the MSiNP and MSiNP-Ag⁺ groups. Source: Reprinted with permission from Royal Society of Chemistry, Chen X, Zhu S, Hu X, et al. Toxicity and mechanism of mesoporous silica nanoparticles in eyes. *Nanoscale*. 2020;12(25):13637–13653. permission conveyed through Copyright Clearance Center, Inc.⁸⁰ **(B)** SiNPs decreased the activities of SOD and GSH-Px in a dose-dependent manner in the aortic arch of Sprague–Dawley rats after 30 days of exposure via intratracheal instillation. ** $P < 0.01$. Source: Reprinted with permission from Feng L, Yang X, Liang S, et al. Silica nanoparticles trigger the vascular endothelial dysfunction and prethrombotic state via miR-451 directly regulating the IL6R signaling pathway. *Part Fibre Toxicol*. 2019;16(1):16. (<http://creativecommons.org/licenses/by/4.0/>).⁶⁶ **(C)** TEM image of mitochondrial morphology after exposure to SiNPs (50 $\mu\text{g/mL}$, 24 h). More aberrantly shaped mitochondria were observed (red arrow) in SiNPs group. The mitochondria in the SiNPs group were mainly short rod-shaped. Scale bars: 2 μm or 500 nm. Source: Reprinted with permission from Royal Society of Chemistry, Qi Y, Ma R, Li X, et al. Disturbed mitochondrial quality control involved in hepatocytotoxicity induced by silica nanoparticles. *Nanoscale*. 2020;12(24):13034–13045. permission conveyed through Copyright Clearance Center, Inc.⁸⁴ MSiNPs, mesoporous SiNPs; MSiNPs-Ag⁺, silver ion-adsorbed mesoporous SiNPs; SOD, superoxide dismutase; GSH-Px, glutathione peroxidase; hCECs, primary human corneal epithelial cells; Mt, mitochondrion; ER, endoplasmic reticulum; Av, autophagic vacuole.

DNA demethylation is mediated by DNA demethylases, which are referred mainly to as the TET protein family.⁹⁹ The TET family includes TET1, TET2, and TET3. Among them, TET1 can promote the oxidation of 5mC to 5-hydroxymethylcytosine (5-hmC). Prenatal high-dose exposure to food-grade SiNPs resulted in a 1.8-fold upregulation

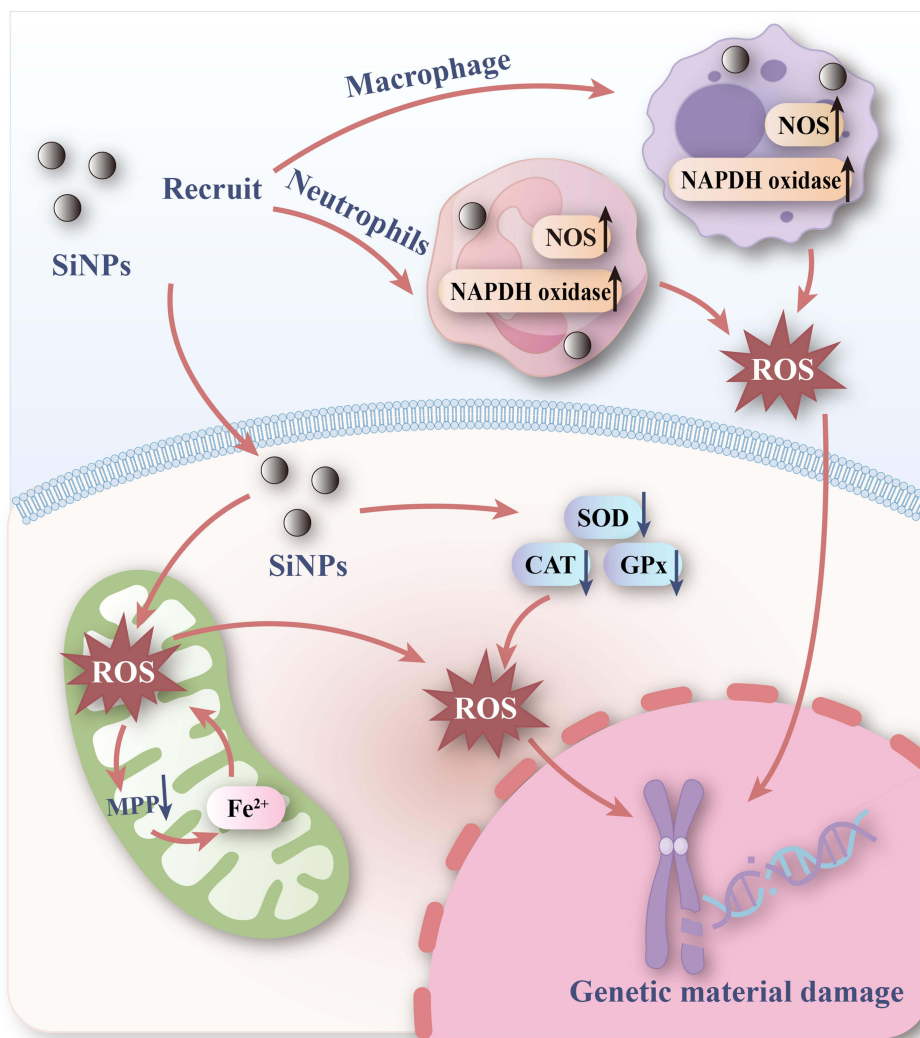


Figure 7 Mechanisms of genetic material damage induced by SiNPs. SiNPs damage genetic material through oxidative stress. On the one hand, SiNPs enter the cell, cause the mitochondria to produce ROS, and lead to the depletion of the antioxidant defense system in the cell, causing damage to the genetic material. On the other hand, SiNPs recruit immune cells such as macrophages and neutrophils to produce ROS, and the release of large amounts of ROS causes damage to genetic material.

of TET1 expression in the maternal liver, whereas low-dose and high-dose exposure significantly downregulated TET1 expression levels in fetal liver tissue.⁶⁰ At present, the specific mechanism by which SiNPs regulate TET1 is still unclear. Furthermore, no studies have reported the effects of SiNPs on the expression of TET2 and TET3, and future research on the mechanism of SiNP-induced DNA methylation is needed.

In addition to regulating related enzymes, SiNPs may block DNA as a substrate for DNMTs, resulting in abnormal DNA methylation. The ROS generated by SiNPs attack DNA to form 8-hydroxy-2'-deoxyguanosine (8-OHdG), which inhibits DNA methylation of nearby cytosine bases to induce DNA hypomethylation. Another type of DNA oxidative damage product, 5hmC, can promote DNA demethylation, leading to DNA hypomethylation.¹⁰⁰ Inflammation caused by SiNPs damages DNA,^{101–103} and damage products such as 5-chlorocytosine and 5-bromocytidine stimulate 5-meC to increase the binding of methyl-binding proteins, thus promoting abnormal hypermethylation.¹⁰⁴

Regulatory Mechanism of microRNA Expression

miRNAs cause gene silencing by inhibiting mRNA translation or degrading mRNAs.¹⁰⁵ Posttranscriptional regulation of gene expression by miRNAs is one of the key mechanisms of the epigenetic toxicity of SiNPs. In the nucleus, miRNA genes are transcribed into pri-miRNAs. Pri-miRNAs are processed in the nucleus and cytoplasm to ultimately produce mature

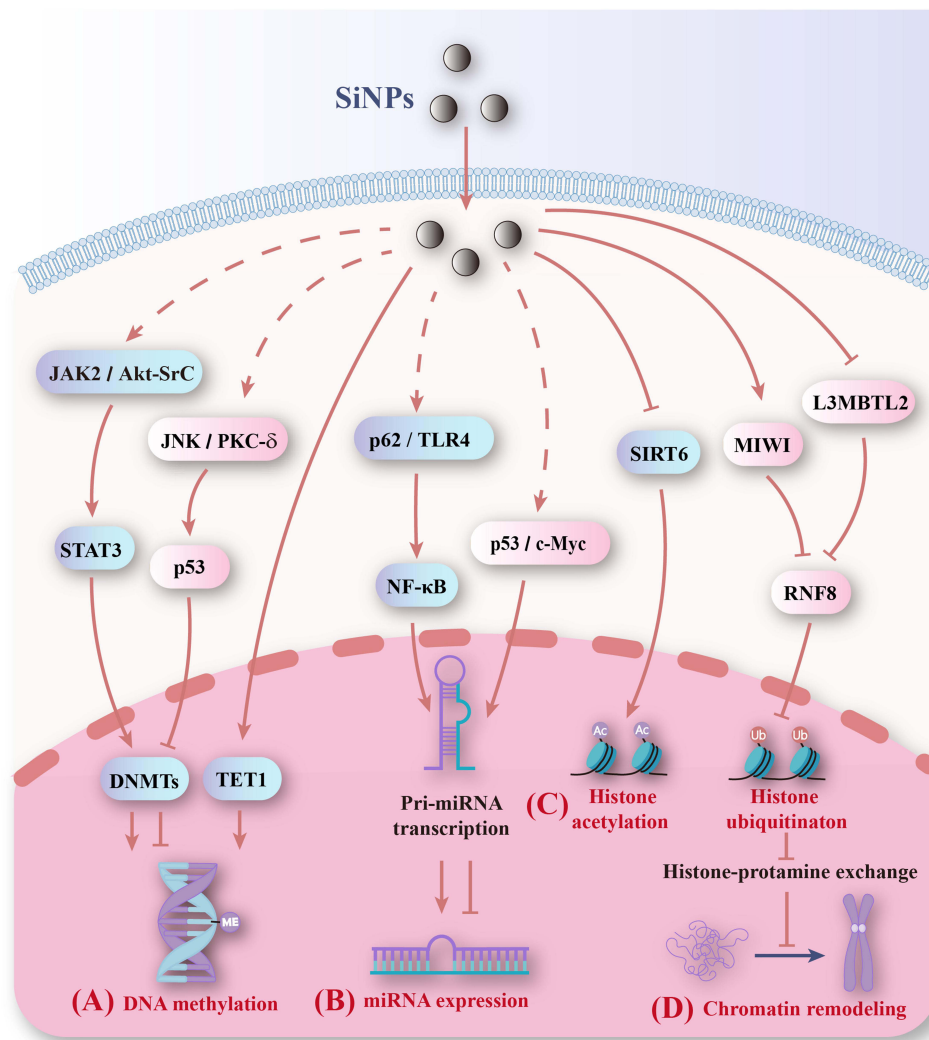


Figure 8 Mechanisms of epigenetic changes induced by SiNPs. (A) SiNPs alter DNA methylation levels by regulating DNMT expression. (B) SiNPs affect the transcription of miRNA genes by regulating transcription factors and subsequently altering miRNA expression. (C) SiNPs promote histone acetylation by decreasing SIRT6 and inhibit histone ubiquitination by reducing RNF8. (D) SiNPs inhibit chromatin remodeling by inhibiting histone ubiquitination.

miRNAs.¹⁰⁶ SiNPs may affect the transcription of miRNA genes by regulating transcription factors and subsequently altering the expression of miRNAs (Figure 8). These transcription factors may include NF- κ B, p53 and c-Myc.

NF- κ B regulates the expression of various miRNAs, such as by promoting the transcription of miRNA-146 and miRNA-21 and inhibiting the transcription of miRNA-29 and miRNA let-7.¹⁰⁷ SiNPs activate NF- κ B through p62 or TLR4. In BEAS-2B cells, SiNPs not only damage lysosomes and block autophagic flux but also stimulate Nrf2 translocation to the nucleus, where it binds to the p62 promoter and activates p62 transcription. These two mechanisms lead to the accumulation of p62, activating the p62/TRAF6/NF- κ B pathway.^{108,109} Moreover, amorphous SiNPs promote the translocation and release of HMGB1 from the nucleus to the cytoplasm and the expression of TLR4 in HUVECs. Subsequently, TLR4 binds to HMGB1, increasing the expression of MyD88 and ultimately activating the NF- κ B signaling pathway.¹⁰²

Exposure to SiNPs can induce p53 activation.⁹⁸ p53 regulates the transcription of miRNAs, such as those in the miRNA-34 family, and promotes Drosha-mediated editing of pri-miRNAs into precursor miRNAs.¹¹⁰ c-Myc regulates the transcription of miRNAs, including miRNA-17 and miRNA-34.¹¹¹ Amorphous SiNP NM-203 has been shown to

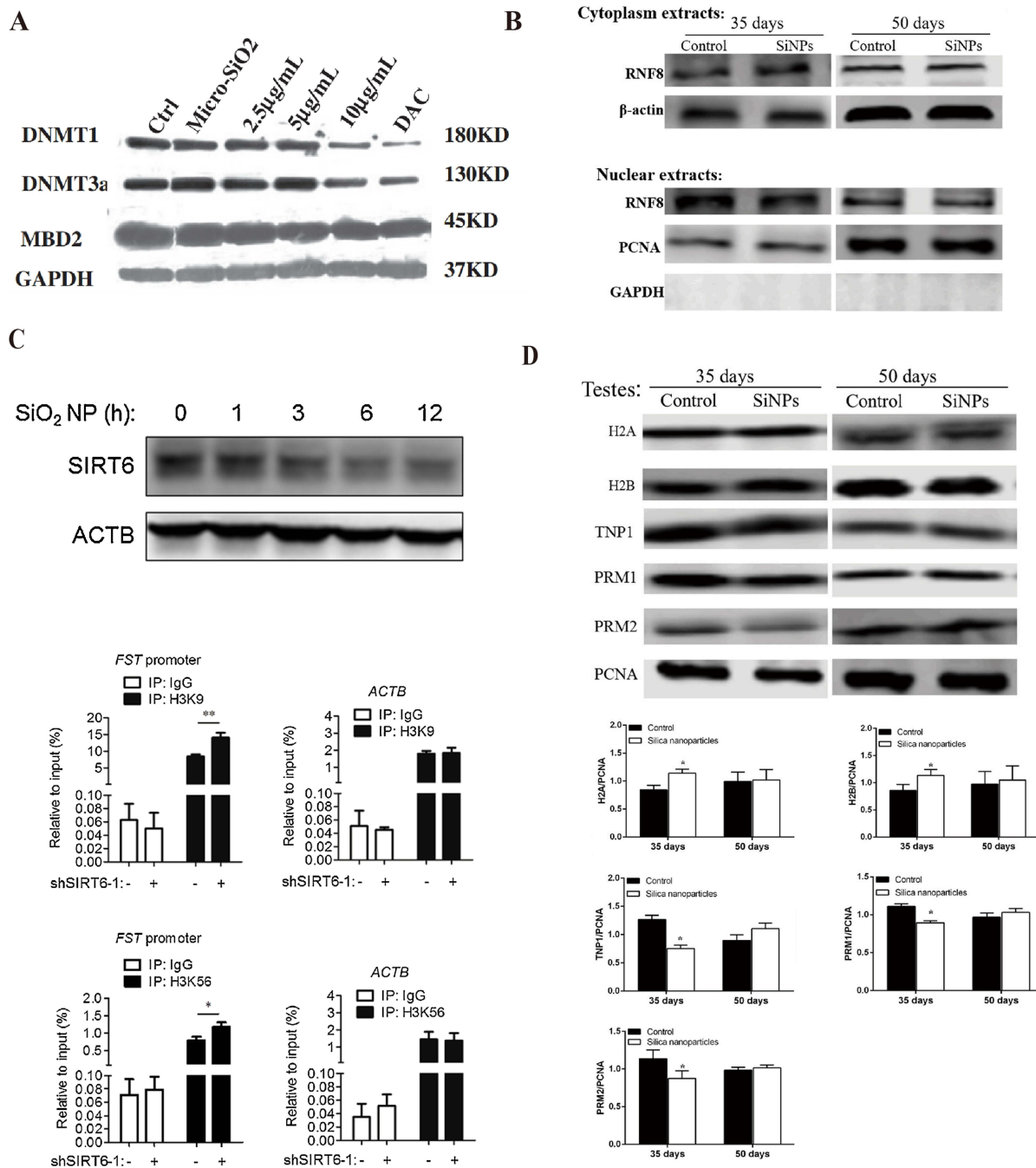


Figure 9 SiNPs induce epigenetic changes by regulating enzymes. **(A)** 15-nm SiNPs (10µg/mL, 24 h) exposure decreased DNMT1 and DNMT3a protein levels in HaCaT cells. Source: Reprinted from Gong C, Tao G, Yang L, Liu J, Liu Q, Zhuang Z. SiO₂ nanoparticles induce global genomic hypomethylation in HaCaT cells. *Biochem Biophys Res Commun.* 2010;397(3):397–400. Copyright 2010, with permission from Elsevier.⁸⁹ **(B)** SiNPs (20 mg/kg.bw) exposure for 35 days inhibited RNF8 expression in nuclear extracts of testes. After the 15-day withdrawal period, the RNF8 levels recovered. Source: Reprinted from Liu J, Li X, Zhou G, et al. Silica nanoparticles inhibiting the differentiation of round spermatid and chromatin remodeling of haploid period via MIWI in mice. *Environ Pollut.* 2021;284:117446. Copyright 2021, with permission from Elsevier.⁶⁹ **(C)** SiNPs (50 µg/mL) decreased SIRT6 protein expression in A549 cells. A549 cells were infected with a virus expressing shRNA targeting SIRT6 or control shRNA. ChIP analysis was performed with antibodies against Ac-H3K9, Ac-H3K56 or control IgG and analyzed by qPCR. SIRT6 reduced Ac-H3K9 and Ac-H3K56 levels at the FST promoter region. The ACTB gene was used as a control. **P* < 0.05, ***P* < 0.01. Source: Reprinted from Zhang L, Han B, Xiang J, Liu K, Dong H, Gao X. Silica nanoparticle releases SIRT6-induced epigenetic silencing of follistatin. *Int J Biochem Cell Biol.* 2018;95:27–34. Copyright 2018, with permission from Elsevier.⁷⁰ **(D)** SiNPs (20 mg/kg.bw) altered the levels of histones and protamine. On day 35 after the first dose, histones (H2A, H2B, H3, H4) were dramatically upregulated, and TNP1, PRM1 and PRM2 were downregulated in the SiNP group. After the 15-day withdrawal period, the injury was reversed. n=5 for each group. **P* < 0.05. Source: Reprinted from Liu J, Li X, Zhou G, et al. Silica nanoparticles inhibiting the differentiation of round spermatid and chromatin remodeling of haploid period via MIWI in mice. *Environ Pollut.* 2021;284:117446. Copyright 2021, with permission from Elsevier.⁶⁹ Micro-SiO₂, microsilized SiNPs; DAC, 5-aza-deoxycytidine, a DNA methyltransferase inhibitor; DNMT, DNA methyltransferase; MBD2, methyl-CpG binding protein 2; RNF8, ring finger protein 8; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; SIRT6, sirtuin 6; ACTB, beta-actin; FST, follistatin; A549 cells, lung epithelial cells; ChIP, chromatin immunoprecipitation; Ac-H3K9, acetylated H3K9; Ac-H3K56, acetylated H3K56.

increase the mRNA and protein levels of c-Myc, possibly by triggering the transcription of the P1 promoter of the c-Myc gene. However, low concentrations of NM-203 increased c-Myc protein levels but did not affect mRNA expression levels.¹¹² This finding indicates that other mechanisms may be involved. SiNPs may also promote c-Myc expression by activating p38 MAPK or ERK1/2.^{36,113,114}

Regulatory Mechanisms of Histone Modification and the Inhibition of Chromatin Remodeling

As mentioned earlier, the histone modifications caused by SiNPs include ubiquitination, phosphorylation, and acetylation. Ring finger protein 8 (RNF8) is a ubiquitin ligase, E3, that regulates the ubiquitination of H2A and H2B and promotes the exchange of histones with protamines.¹¹⁵ SiNPs led to a reduction in lethal (3) malignant brain tumor-like 2 (L3MBTL2) levels, which inhibited RNF8-ubH2A/ubH2B and affected spermatogenesis.³⁵ Moreover, SiNPs can reduce RNF8 in the sperm nucleus by increasing PIWI-like protein 1 (MIWI) expression or inhibiting the degradation of MIWI (Figure 9B).⁶⁹ SiNPs may also activate S-phase kinase associated protein (Skp2). Skp2 is also an important E3 ubiquitin ligase. Akt and AMPK activated by SiNPs are upstream regulatory molecules of Skp2.^{116–119} These findings suggest that Skp2 may be one of the mechanisms by which SiNPs regulate histone ubiquitination.

Histone acetylation is mediated by histone acetyltransferase (HAT) and histone deacetylase (HDAC). Sirtuin 6 (SIRT6) is an H3K9 and H3K56 hDAC that can regulate the deacetylation of histones. Research has shown that the overexpression of SIRT6 leads to a decrease in H3K9 and H3K56 acetylation levels.¹²⁰ Exposure to SiNPs has been shown to inhibit SIRT6 expression and increase the acetylation levels of H3K9 and H3K56 at the follistatin (FST) gene promoter region (Figure 9C). Further research revealed that SiNPs shorten the half-life of SIRT6 mRNA without altering the activity of the SIRT6 promoter.⁷⁰ These findings suggest that SiNPs may reduce the expression of SIRT6 through a posttranscriptional regulatory mechanism. SiNPs may play a posttranscriptional regulatory role in SIRT6 by activating PI3K. A previous study reported that SiNPs activate the PI3K pathway.¹²¹ PI3K activation promotes miRNA-34a expression. miRNA-34a binds to the 3'-UTR of SIRT6 mRNA, reducing its stability and leading to its degradation.¹²²

SiNPs inhibit chromatin remodeling by inhibiting histone ubiquitination (Figure 8). The ubiquitination of H2A and H2B promotes the removal of histones, which is beneficial for replacing histones with histone-protamines. SiNPs inhibited histone-protamine exchange by decreasing H2A and H2B ubiquitination (Figure 9B and D), resulting in the inhibition of chromatin remodeling.^{35,69} Histone acetylation also regulates chromatin remodeling. Histone variant hyperacetylation is beneficial for chromatin remodeling. During the process of histone-protamine exchange, histones are first replaced by histone variants. Histone variant hyperacetylation leads to the unwinding of nucleosome DNA, resulting in a loose chromatin structure. Subsequently, topoisomerase II beta causes DNA breaks, promotes the removal of histone variants, and is replaced by transition proteins.⁷⁴ NPs can reduce the acetylation level of histones. For example, zinc oxide nanoparticles reduce the acetylation level of histone H4K5.¹²³ Nickel nanoparticles reduce acetylation levels of histone H3 in human bronchial epithelial cells.¹²⁴ A previous study reported that SiNPs alter histone H3K9 and H3K56 acetylation levels, but their effects on the acetylation levels of histone variants are still unclear.⁷⁰ These findings indicate that SiNPs may inhibit chromatin remodeling by inhibiting histone variant acetylation, but further research is needed for verification.

Factors Influencing the Genetic and Epigenetic Toxicity of SiNPs

Particle Size

Particle size is one of the key factors affecting the genetic and epigenetic toxicity of SiNPs. Microsized SiNPs (1–5 μm) do not induce changes in DNA methylation levels in HaCaT cells, whereas 15-nm SiNPs reduce global DNA methylation levels.⁸⁹ The genotoxicity of SiNPs increases with decreasing particle size. Amorphous SiNPs of four sizes (10, 25, 50, and 100 nm) were used to study the genotoxicity of SiNPs in human umbilical vein endothelial cells. The results indicated that the smaller the particle size was, the greater the induced genotoxicity.⁴⁵ This may be because smaller particles have a larger surface area per unit mass, and their reactivity increases accordingly, which can produce more ROS at a given mass.¹²⁵ The cellular uptake efficiency of SiNPs of different sizes is different. Smaller NPs are more likely to penetrate the cell

membrane and cause cell damage.^{126,127} Larger than 100 nm SiNP have been proven to have good biocompatibility and not induce genotoxicity.¹²⁸ In addition, small SiNPs may aggregate into larger agglomerated particles and cause severe genotoxicity.¹²⁹ Therefore, SiNPs induce genetic and epigenetic toxicity in a size-dependent manner.

Dose

The genetic and epigenetic toxicity induced by SiNPs is dose dependent.¹³⁰ With increasing doses of SiNPs, the genetic and epigenetic toxicity effects increase. SiNPs inhibited the ubiquitination of histones H2A and H2B in a dose-dependent manner in the sperm of mice.³⁵ SiNPs induce DNA breakage in rat lymphocytes in a linear dose-dependent manner.¹³¹ Similarly, the highest concentration of SiNPs (100 µg/mL) induced the most significant DNA damage in onion tissue cells.⁴² This occurs because the higher the concentration of NPs is, the greater the degree of cellular uptake. After passing through the cell membrane, NPs are localized mainly in lysosomes. When excessive NPs accumulate, cells eliminate excess NPs through extracellular secretion and lysosomal escape mechanisms.^{132,133} However, when the amount of NPs exceeds a certain threshold, the NPs accumulate in the extracellular matrix and damage the cell membrane, leading to lysosomal rupture due to lysosomal overload. This damage ultimately leads to cytotoxicity and cell death.¹³²

Geometric Shape

SiNPs are spherical, rod shaped, plate shaped, etc. The geometric shape of SiNPs affects cell internalization. Compared with spherical mesoporous SiNPs, rod-shaped mesoporous SiNPs induce more severe genotoxicity;⁵² this may be due to the high aspect ratio of rod NPs, which are taken up by cells faster and in greater quantities.¹³⁴ The use of mesoporous SiNPs with different aspect ratios also revealed that particle shape affected the development of actin tissue and filopodia in HeLa cells.¹³⁵ In addition, at the same volume, rod-shaped NPs have a larger surface area than spherical NPs do, and the surface area available for interaction and contact with the cell membrane is larger, making them easier to absorb by cells and causing more severe genotoxicity.¹³⁶ The shape of SiNPs also affects their biological distribution and clearance rate. Spherical mesoporous SiNPs are easily retained in the liver, whereas long rod-shaped mesoporous SiNPs are easily distributed in the spleen.¹³⁷ The clearance rate of short rod-shaped mesoporous SiNPs is faster than that of long rod-shaped mesoporous SiNPs.¹³⁸ This finding indicates that SiNPs with a longer aspect ratio have a longer residence time in the body and greater genotoxic effects. At present, it is not clear whether the shape of SiNPs affects the magnitude of epigenetic toxicity.

Structure Type

Crystalline SiNPs are arranged in a regular manner, whereas amorphous SiNPs are arranged irregularly. Different types of SiNPs have different parameters, such as morphology, porosity and crystallinity, which affect the biological distribution and genetic and epigenetic toxicity of SiNPs.¹³⁹ Generally, the genetic and epigenetic toxicity effects of crystalline SiNPs are greater than those of amorphous SiNPs, possibly because the physical and chemical properties are affected by the crystal structure.^{112,140} In addition, the toxicity of mesoporous SiNPs has been shown to be significantly greater than that of nonporous SiNPs. This occurs because the density of mesoporous SiNPs is lower, and therefore, the weight of each particle is lower, resulting in a greater number of particles per unit mass.¹⁴¹

Administration Route

SiNPs can enter the human body through intravenous injection, airway inhalation, skin contact, etc. Different administration routes affect the biological distribution and local tissue concentration of SiNPs, leading to different genetic and epigenetic toxicity effects. After oral or intravenous injection, SiNPs are distributed mainly in the liver or spleen. Through tracheal instillation, they are distributed mainly in lung tissue.¹³⁹ Different biological distributions result in different types of cells being exposed to SiNPs, thus affecting genotoxicity. Cabellos et al studied the genotoxicity of the oral administration of nonporous and mesoporous SiNPs and reported that DNA in several organs and tissues of rats was not damaged.¹⁴² Similarly, studies on intratracheal instillation have not shown genotoxic effects of amorphous SiNPs (15 nm and 55 nm in size).¹⁴³ However, in a study by Downs et al, SiNPs (15 nm and 55 nm in size) caused DNA damage in the liver and micronucleus when injected intravenously.¹⁴⁴ This may be because the concentration of SiNPs that reach local tissues via the intravenous route is greater than that via tracheal drip or the oral route.

Surface Charge

Compared with negatively charged SiNPs, SiNPs with a positive surface charge cause greater genotoxicity.¹⁴⁵ On the one hand, positively charged NPs are more easily internalized by cells. The cell membrane is negatively charged because of the phospholipid layer on the outer surface. NPs with positive charges are more likely to enter cells through the cell membrane due to electrostatic interactions.¹⁴⁶ On the other hand, positively charged SiNPs are more likely to interact with negatively charged DNA, causing damage to genetic material.¹³⁸ Therefore, SiNPs with positive charges are more likely to penetrate the cell membrane, bind to negatively charged DNA, and damage DNA. However, whether surface charge of SiNPs cause epigenetic toxicity is unclear.

Other Factors

The genetic and epigenetic toxicity effects induced by SiNPs may also be related to the exposure time of the particles and the type of toxicity tested. The longer the exposure time is, the greater the dose of NPs reaching the local tissues of the organism, and the corresponding prolongation of contact time with cells results in greater genetic and epigenetic toxicity. In addition, the type of experiment can affect the experimental results. In the Ames test, spherical amorphous SiNPs were shown not to induce gene mutations.¹⁴⁷ This is because not all particles can pass through bacterial membranes; thus, the Ames test may not be suitable for evaluating the genotoxicity of NPs. Therefore, evaluating the genotoxicity of SiNPs also requires consideration of the type of experiment.

Limitations and Future Outlook

The genetic and epigenetic toxicity of SiNPs has received increasing attention, especially their adverse effects on the reproductive system. The experimental data on the genetic and epigenetic toxicity of SiNPs to the reproductive system have been mostly derived from animal models, with a lack of actual human exposure and epidemiological data. Currently, deriving impacts on human health from animal data is not possible. In the future, emphasis should be placed on transforming basic toxicology research results into medical indicators for measuring human risk. In addition, most studies are based on the results of short-term exposure (less than 3 months) to SiNPs. Long-term in vivo exposure experiments are needed to accurately predict harm to the human body and adverse consequences for offspring.

There are inconsistent or even contradictory results regarding the genetic and epigenetic toxicity of SiNPs, which may depend on differences in particle properties (such as size, structure, and shape), exposure status (such as exposure pathway, dose, and time), and cell type. Therefore, when evaluating genetic and epigenetic toxicity, a thorough physical and chemical characterization of SiNPs, including size, surface area, shape, crystallinity, porosity, surface charge, agglomeration, etc., is needed. Second, differences in evaluation methods should be considered to avoid false-negative results. It is necessary to establish a standard guideline for evaluation. In addition, a comprehensive and complete evaluation of the epigenetic changes induced by SiNPs is needed. Owing to the ability of SiNPs to induce multiple abnormal epigenetic changes, an aim of future research should be to simultaneously detect multiple types of epigenetic changes, including at least DNA methylation, miRNA expression changes, and histone modifications.

Exploring the potential mechanisms of SiNPs-induced genetic and epigenetic toxicity will be beneficial for developing new treatment strategies and reducing the adverse effects of SiNPs genetic and epigenetic toxicity on human health. However, there are still many gaps in our understanding of how SiNPs induce epigenetic toxicity. In the future, it will be necessary to further explore the potential molecular mechanisms by which SiNPs induce changes in miRNA expression, such as how SiNPs affect the maturation and processing of miRNAs. The effects of SiNPs on enzymes related to histone modification, such as E1, E2 and HAT, as well as how to regulate chromatin remodeling processes through histone modification, are all worthy of attention.

Avoiding and reducing toxicity risk are urgent tasks. First, an assessment standard for SiNPs should be established. The best testing and testing conditions should be selected to increase the likelihood of extrapolating genetic and epigenetic toxicity results to human risk. Second, safe and nontoxic SiNPs should be designed and developed to reduce genetic and epigenetic toxicity: (1) the size, shape, and structure of SiNPs should be optimized; (2) by doping, metal loading should be used to reduce the oxidative stress damage caused by SiNPs; and (3) the

surface chemical properties and in vivo clearance rate of SiNPs should be modified through functional group surface modification. Third, researchers should focus on the key targets of genetic and epigenetic toxicity mechanisms. For example, key mechanistic targets for regulating the expression of miRNAs, such as NF- κ B and p53, have been identified. The development of drugs that inhibit these pathways in clinical practice and block key components of these pathways, such as upstream stimuli or downstream targets, could be used to treat SiNP genetic and epigenetic toxicity. These methods provide broader application prospects for SiNPs and can improve their application in the biomedical field.

Conclusion

The genetic and epigenetic toxicity induced by SiNPs, especially damage to germ cells, cause a risk of infertility and teratogenesis. SiNPs induce genetic material damage, which manifests as gene mutations, DNA strand breaks, and chromosomal aberrations. The main mechanism is oxidative stress damage mediated by mitochondrial dysfunction and inflammation. Furthermore, the molecular mechanisms of epigenetic toxicity are discussed in detail for the first time, SiNPs alter DNA methylation, miRNA expression, histone modification and inhibit chromatin remodeling by regulating epigenetic-related enzymes and transcription factors. The potential solutions to avoid toxicity were prospected and provide a guidance for better application of SiNPs in the biomedical field. In the future, it will be necessary to focus on the key targets of genetic and epigenetic toxicity mechanisms. How to reduce the genetic and epigenetic risk or toxicity of SiNPs is still challenging, and material modification or the synthesis of safe SiNPs is urgently needed.

Acknowledgments

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Disclosure

The authors report no conflicts of interest in this work.

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