



## Review or mini-review

Contribution of oxidative stress to TiO<sub>2</sub> nanoparticle-induced toxicityBin Song<sup>a,b</sup>, Ting Zhou<sup>a</sup>, WenLong Yang<sup>a</sup>, Jia Liu<sup>b</sup>, LongQuan Shao<sup>b,\*</sup><sup>a</sup> Guizhou Provincial People's Hospital, Guiyang 550002, China<sup>b</sup> Nanfang Hospital, Southern Medical University, Guangzhou 510515, China

## ARTICLE INFO

## Article history:

Received 2 August 2016

Received in revised form 14 October 2016

Accepted 15 October 2016

Available online 18 October 2016

## Keywords:

Titanium dioxide nanoparticles

Apoptosis

Inflammatory response

DNA methylation

DNA damage oxidative stress

## ABSTRACT

With the rapid development of nanotechnology, titanium dioxide nanoparticles (TNPs) are widely used in many fields. People in such workplaces or researchers in laboratories are at a higher risk of being exposed to TNPs, so are the consumers. Moreover, increasing evidence revealed that the concentrations of TNPs are elevated in animal organs after systematic exposure and such accumulated TNPs could induce organ dysfunction. Although cellular responses such as oxidative stress, inflammatory response, apoptosis, autophagy, signaling pathways, and genotoxic effects contribute to the toxicity of TNPs, the interrelationship among them remains obscure. Given the pivotal role of oxidative stress, we summarized relevant articles covering the involvement of oxidative stress in TNPs' toxicity and found that TNP-induced oxidative stress might play a central role in toxic mechanisms. However, available data are far from being conclusive and more investigations should be performed to further confirm whether the toxicity of TNPs might be attributed in part to the cascades of oxidative stress. Tackling this uncertain issue may help us to comprehensively understand the interrelationship among toxic cellular responses induced by TNPs and might shed some light on methods to alleviate toxicity of TNPs.

© 2016 Elsevier B.V. All rights reserved.

## Contents

1. Introduction .....	130
2. Bio-distribution of TNPs after systematic exposure .....	131
3. Oxidative stress as a common cellular response contributed to the toxicity of TNPs .....	132
3.1. <i>In vivo</i> studies regarding the role of OS in TNP-induced toxicity .....	132
3.2. <i>In vitro</i> studies regarding the role of OS in TNP-induced toxicity .....	133
4. Potential molecular mechanisms by which TNPs regulate oxidative stress .....	134
5. Cellular responses possibly initiated by TNP-induced OS .....	135
5.1. <i>In vivo</i> studies .....	136
5.2. <i>In vitro</i> studies .....	137
6. Conclusion .....	138
Competing interests .....	138
Acknowledgements .....	138
References .....	138

## 1. Introduction

Titanium dioxide nanoparticles (TNPs), less than 100 nm in size, are extensively applied in many fields such as environmental application (Li et al., 2008), battery production (Deng et al., 2009), bio-medical industry (Singh and Nalwa, 2011), food and personal care appliances (Weir et al., 2012), construction industry (Sang et al., 2014), and cosmetics (Lu et al., 2015), due to their splendid physicochemical characteristics such as anti-bacterial, anti-ultraviolet, photocatalytic, and self-cleaning

\* Corresponding author at: Department of Stomatology, Nanfang Hospital, South Medical University, China.

E-mail addresses: [17055224@qq.com](mailto:17055224@qq.com) (B. Song), [zhouting187@126.com](mailto:zhouting187@126.com) (T. Zhou), [18985147600@163.com](mailto:18985147600@163.com) (W. Yang), [liujia1988dr@163.com](mailto:liujia1988dr@163.com) (J. Liu), [shaolongquan@smu.edu.cn](mailto:shaolongquan@smu.edu.cn) (L. Shao).

properties (Montazer and Seifollahzadeh, 2011). TNPs can be classified into anatase and rutile types (Chen and Mao, 2007). Each of which possess subtle different physicochemical properties, resulting in different toxicities. It was reported that the toxicity of anatase TNPs was higher than that of rutile TNPs (Clemente et al., 2015). However, the rapid development of nanotechnology acts as a double-edged sword. Widespread usage indicated that the unintentional exposure risk was significantly promoted. People in such workplaces or researchers in laboratories are at a high risk of being exposed to TNPs (Silva et al., 2015; Pelclova et al., 2016), so are the consumers (Hsu and Chein, 2007). Besides, a large number of *in vivo* studies revealed that the TNP contents are elevated in the animal brain, liver, lung, spleen, heart, and kidney after exposure, and such accumulation, in turn, exhibits toxic effects on these organs, leading to organ dysfunction. Moreover, perinatal exposure to TNPs can impair fetal development (Mohammadipour et al., 2014, 2016). In addition, numerous *in vivo* and *in vitro* studies demonstrated that TNPs could upregulate the proportion of apoptotic cells (Marquez-Ramirez et al., 2012), regulate the cell skeleton (Gheshlaghi et al., 2008; Mao et al., 2015), induce oxidative stress (OS) (Hu et al., 2015), inhibit cell cycle (Kansara et al., 2015), activate signaling pathways (Neacsu et al., 2015), induce an inflammatory response (Giovanni et al., 2015), and lead to dysregulated autophagy (Lopes et al., 2016), all of which might probably contribute to TNP toxic effects in several primary cells or cell lines. Overall, increasing evidence confirmed the toxic effects of TNPs, which suggests that TNP exposure might become a serious threat to human health.

However, the correlation among cellular responses such as OS, apoptosis, autophagy, inflammatory response, and signaling pathways, which contribute to the toxicity of TNPs, remain unclear. Among them, OS can be simply defined as a status of excessive reactive oxygen species (ROS) and reactive nitrogen species (RNS) production beyond the scavenging capabilities of anti-oxidants (Jones, 2006). OS status is mainly determined by ROS and RNS levels, the activities of enzymatic anti-oxidants, and the contents of non-enzymatic anti-oxidants. Meanwhile, OS is associated with several cellular responses (Fig. 1), and it is generally accepted as an important pathogenic factor in many human diseases such as Alzheimer's disease (Zawia et al., 2009), periodontitis (Miricescu

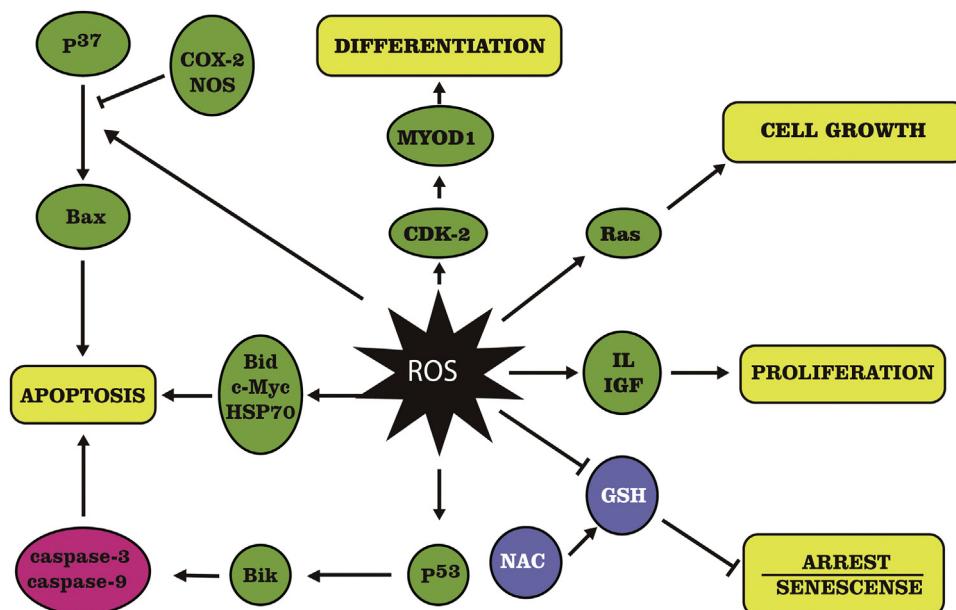
et al., 2014), metabolic syndrome (Bonomini et al., 2015), and cancer (Ahmadi and Shadboorestan, 2016). Moreover, OS contributes to the toxic effects of exogenous insults (Yang and Omaye, 2009). Therefore, given the important role of OS in many cellular responses and in toxic effects of exogenous stimuli, we summarized relevant articles about the involvement of OS in TNPs' toxicity and discussed whether other cellular responses induced by TNPs were the cascades of oxidative stress. Tackling such uncertain issue might help us to improve the bio-safety of TNPs enabled products.

## 2. Bio-distribution of TNPs after systematic exposure

Widespread applications of TNP-enabled products increase the risk of being exposed. Workers, researchers, and consumers might be exposed to TNPs unintentionally. A recent report (Pelclova et al., 2016) showed that the TNP content in the exhaled breath condensate (EBC) was significantly higher in production workers in TNP workplaces, which suggested that inhalation exposure could upregulate TNP contents in humans. However, most data about the bio-distribution of TNPs were obtained from *in vivo* studies, which will be illustrated below.

The contents of TNPs in the rat spleen, lung, and liver remain at higher levels several days after intravenous administration (Fabian et al., 2008; van Ravenzwaay et al., 2009; Shinohara et al., 2014; Elgrabli et al., 2015). Umbreit et al. (2012) also found that the levels of TNPs in the mouse liver, kidney, brain, lung, heart, lymph node, and spleen were still high several weeks after TNPs exposure through intravenous injection.

In addition to intravenous injection, intranasal administration also upregulated TNP contents in the mouse brain and liver (Wang et al., 2008a). TNP concentrations in the mouse spleen, lung, kidney, and liver were still at high levels 14 days after intraperitoneal injection (Chen et al., 2009). Meanwhile, TNP contents in the mouse liver, kidney, spleen, lung, brain, and heart were enhanced after abdominal injection (Liu et al., 2009). In addition, TNP concentrations in the rat lung, liver, kidney, spleen, brain, small intestine, testicles, heart, and blood were upregulated after intragastric administration (Hendrickson et al., 2016).



**Fig. 1.** Roles of ROS in cellular responses including apoptosis, differentiation, cell growth, proliferation, and arrest senescence (Mates et al., 2008). CDK-2: cyclin-dependent kinase 2; COX-2: cyclooxygenase-2; GSH: glutathione; HSP70: 70 kDa heat shock protein; IGF: insulin-like growth factor; IL: interleukine; NAC: N-Acetyl-L-Cysteine; NF- $\kappa$ B: necrosis factor kappa B; NOS: nitric oxide synthase; ROS: reactive oxygen species.

TNPs can be observed in fetuses after perinatal exposure, which suggest that TNPs might induce developmental toxicity. Yamashita et al. (2011) found that the TNP content in the placenta and fetal brain increased when pregnant mice were exposed to TNPs through intravenous injection. After pregnant rats were treated with TNPs through intragastric administration, TNP concentration in the hippocampus of one-day-old neonates was also upregulated (Mohammadipour et al., 2014).

The bio-distribution of TNPs depends on different administration routes. Patri et al. (2009) revealed that subcutaneous injection increased TNPs contents in the mouse spleen and liver. However, in addition to the spleen and liver, intravenous injection upregulated TNP concentrations in the mouse lung and kidney one day after treatment. Although oral exposure did not increase TNP contents in rat organs, intravenous injection increased TNP concentrations in the rat liver, spleen, kidney, lung, heart, brain, thymus, and reproductive organs several days after exposure (Geraets et al., 2014).

The above-mentioned studies suggest that an increase in TNP levels could be observed in major organs after systematic administration (Table 1). Meanwhile, TNP contents remained higher even several days after exposure, which indicated that TNPs would be gradually deposited in organs. This accumulation might induce toxic effects, leading to organ impairments. Meanwhile, bio-distribution after inhalation should be explored in depth for mimicking occupational exposure. Moreover, bio-distribution of TNPs in the fetus during perinatal exposure should be comprehensively investigated.

### 3. Oxidative stress as a common cellular response contributed to the toxicity of TNPs

A recent survey (Pelclova et al., 2016) showed that OS-related biomarkers are upregulated in EBC collected from production workers in TNP workplace, which suggested that TNPs can induce OS in humans. However, most conclusions about the role of OS in the toxicity of TNPs were obtained from *in vivo* and *in vitro* studies.

#### 3.1. In vivo studies regarding the role of OS in TNP-induced toxicity

Numerous *in vivo* studies disclosed that TNP contents in main organs increased after animals received TNPs through various

administration route, which, in turn, induced OS and dysfunctional organs.

Oral exposure was the common administration routes. Mice exposed to TNPs through drinking water exhibited upregulated 8-hydroxyl deoxyguanosine (8-OHdG) level and DNA damage in the liver (Trouiller et al., 2009). Zhang et al. (2010) also found that after mice were exposed to TNPs through oral gavage, TNP contents in the liver, kidney, cortex, and hippocampus were elevated. Besides, the ROS production increased, accompanied by the inhibition of glutathione peroxidase (GSH-Px) and superoxide dismutase (SOD) activities. Meanwhile, methane dicarboxylic aldehyde (MDA) levels were elevated in the liver, kidney, and hippocampus. Shrivastava et al. (2014) showed that oral exposure to TNPs could increase ROS and glutathione (GSH) levels, and inhibit activities of SOD and catalase (CAT), with a concomitant reduction in CuZnSOD and MnSOD gene expression in the mouse erythrocytes, liver, and brain. Besides, TNPs increased the production of ROS and MDA, accompanied by a reduction in GSH level in the mouse liver after oral administration (Shukla et al., 2014). Hu et al. (2015) treated mice with TNPs through oral administration and TNP-treated mice exhibited decreased total superoxide dismutase (T-SOD) activities, reduced GSH level, and elevated production of MDA in the serum and liver, accompanied by increased plasma glucose, activated mitogen-activated protein kinase (MAPK) pathway, and upregulated levels of pro-inflammatory factors.

In addition to oral exposure, intravenous injection is another major administration route. Meena et al. (2015a) revealed that the TNP content in the rat testis was elevated after intravenous injection, accompanied by inhibition of SOD and GSH-Px activities as well as increased MDA levels. DNA damage, upregulated apoptosis, decreased sperm count, and impaired reproductive function were observed in TNP-treated rats as well. The same group (Meena et al., 2015b) also found that intravenous injection led to elevated TNP content in the rat brain with a concomitant increase in ROS and MDA production, inhibition of SOD and GSH-Px activities as well as downregulated melatonin level. In addition, the inflammatory response was promoted and apoptosis was enhanced in the brain of the TNP-treated animals. Zhang et al. (2015) confirmed that after mouse dams were exposed to TNPs through intravenous injection during the lactation period, TNP contents in the liver, spleen, lung, kidney, and mammary gland were elevated, accompanied by upregulated MDA levels and downregulated GSH expression in the liver and mammary gland. Meanwhile, the levels of tight junction

**Table 1**

Bio-distribution of TNPs after systematic exposure.

Routes of administration	Organ distribution	Ref
Rat intravenous administration	spleen, lung, and liver	Elgrabli et al. (2015), Fabian et al. (2008), Shinohara et al. (2014), van Ravenzwaay et al. (2009)
Mouse intravenous injection	liver, spleen, kidney, lung, heart, brain, thymus, and reproductive organs	Geraets et al. (2014)
Mouse intraperitoneal injection	liver, kidney, brain, lung, heart, lymph node, and spleen	Umbreit et al. (2012)
Mouse abdominal injection	spleen, lung, kidney, and liver	Chen et al. (2009)
Mouse intranasal administration	liver, kidney, spleen, lung, brain, and heart	Liu et al. (2009)
Rat intragastric administration	brain and liver	Wang et al. (2008a)
Mouse subcutaneous injection	lung, liver, kidney, spleen, brain, small intestine, testicles, heart, and blood	Hendrickson et al. (2016)
Mouse intravenous injection	spleen and liver	Patri et al. (2009)
Pregnant rat intragastric administration	Spleen, liver, lung, and kidney	Mohammadipour et al. (2014)
Pregnant mouse intravenous injection	one-day-old neonate hippocampus	
	placenta and fetal brain	Yamashita et al. (2011)

proteins, zonula occludens 1 and occludin, were downregulated, indicating impaired blood–milk barrier, which led to increased TNP contents in pups.

Intranasal instillation is another frequently used administration route. Wang et al. (2008c) reported that intranasal instillation led to increased TNP concentrations and upregulated MDA levels in the mouse brain zones. However, the activities of GSH-Px, glutathione transferase (GST), and SOD were promoted 10 days after exposure, and then declined. The same research group (Wang et al., 2008b) also found that glutamic acid, nitric oxide (NO), and protein carbonyl levels were upregulated, accompanied by increased expression of glial fibrillary acidic protein (GFAP), indicating impairments to the mouse brain 30 days after intranasal administration. Ze et al. (2014) revealed that 90 days nasal administration increased the TNP content in the mouse brain. In turn, this accumulation upregulated the levels of  $O_2^{•-}$ ,  $H_2O_2$ , MDA, carbonyl, and 8-OHdG, and dysregulated mRNA expression of OS-related genes; all of which contributed to histopathological changes in the mouse brain.

Intragastric administration could induce OS in animal organs. Cui et al. (2010) showed that mice exhibited increased levels of  $O_2^{•-}$ ,  $H_2O_2$ , NO, and MDA and downregulated gene expression of antioxidant factors in the liver after intragastric exposure to TNPs, which might contribute to cell apoptosis. Later, the same group (Cui et al., 2012) discovered that intragastric administration of TNPs for 90 days changed OS-related gene expression determined by microarray assay with a concomitant increase in TNP content in the mouse liver. Hu et al. (Hu et al., 2011) confirmed that, after mice were exposed to TNPs through intragastric administration, the TNP content in the mouse hippocampus increased, accompanied by elevated levels of  $O_2^{•-}$  and  $H_2O_2$ , inhibition of SOD, CAT, ascorbate peroxidase (Apx), and GSH-Px activities, and decreased ratio of ascorbic acid (AsA)/oxidized AsA (DAsA) and glutathione (GHS)/oxidized glutathione (GSSG). Meanwhile, cell apoptosis was upregulated in TNP-treated mouse hippocampus. Female mice also exhibited enhanced TNPs content and increased levels of  $O_2^{•-}$ ,  $H_2O_2$ , and 8-OHdG in the ovary after intragastric exposure to TNPs, which was associated with dysfunctional ovary (Gao et al., 2012).

Intratracheal instillation is another option for administrating TNPs. Liang et al. (2009) revealed that TNPs reduced SOD activities in the plasma, liver, and kidney and inhibited glutathione peroxidase (GSH-Px) activity in the kidney after rats were exposed through single intratracheal instillation. In addition, TNP exposure through intratracheal instillation reduced GSH levels, inhibited T-SOD activity, and upregulated the production of MDA and NO in rat bronchoalveolar lavage fluid (BALF), with a concomitant increase in TNF- $\alpha$  expression (Liu et al., 2013).

Other administration routes that are least frequently used could lead to OS as well. Dermal exposure increased the TNP concentrations in the skin, heart, liver, spleen, lung, kidney, and brain of hairless mice. Meanwhile, MDA levels in the skin and liver were enhanced accompanied by the inhibition of SOD activity. Such changes contributed to impairments in these organs (Wu et al., 2009). Meanwhile, intraarticular administration of TNPs not only impaired the rat knee joints, but also induced histopathological alterations in the heart, lung, and liver. In addition, GSH-Px and SOD activities were promoted, with a concomitant increase in GSH,  $H_2O_2$ , MDA and GSSG levels in TNP-treated synovium (Wang et al., 2009). Ma et al. (2010) found that the content of TNPs was enhanced in the mouse brain after exposure through the abdominal cavity. In addition, brain cells presented filamentous shapes and transformed into inflammatory cells. Meanwhile, the contents of  $O_2$ ,  $H_2O_2$ , and MDA increased, accompanied by inhibition of SOD, CAT, and GSH-Px. In addition, the ratios of AsA/DAsA and GSH/GSSG decreased. Nitric oxide synthase (NOS) activity, including cNOS and iNOS, was

promoted, accompanied by elevated NO level and decreased GSH level.

We can draw a conclusion from the above-mentioned studies that after systematic administration, TNP contents in the main organs were upregulated, which, in turn, induced OS, leading to dysfunctional organs. However, for better mimicking occupational exposure, the toxicity of TNPs should be evaluated through inhalation administration in the future.

### 3.2. In vitro studies regarding the role of OS in TNP-induced toxicity

*In vivo* studies demonstrated that OS in the main organs such as the brain, liver, lung, kidney, and spleen was induced after systematic administration of TNPs, which was further confirmed by many *in vitro* studies. Generally, TNPs can be internalized after exposure mainly via endocytosis, phagocytosis, and micropinocytosis. Studies revealed that prostate cancer PC-3M cells (Thurn et al., 2011) and BV-2 microglia cells (Hsiao et al., 2016) could internalize TNPs through clathrin-dependent endocytosis and phagocytosis. Meanwhile, TNPs were taken up by neutrophils (da Rosa, 2013) or macrophages (Sund et al., 2014) via phagocytosis. Moreover, endocytosis was involved in the uptake of TNPs by human HaCaT keratinocytes (Jaeger et al., 2012), A549 cells (Tedja et al., 2012), human cervical cancer cells (HeLa cells) (Zhang et al., 2013), neural stem cells (Wang et al., 2013b), and Caco-2 intestinal cells (Gitrowski et al., 2014). Ultimately, uptake of TNPs by cells could induce toxicity. In addition, TNPs were internalized by U373 cells mainly through micropinocytosis (Huerta-Garcia et al., 2015).

Primary brain cells or cell lines were used for assessing the neurotoxicity of TNPs in most cases. TNPs decreased cell viability in PC12 cells, with a concomitant increase in lactate dehydrogenase (LDH) activity as well as MDA and ROS production. Meanwhile, GSH levels decreased, accompanied by the inhibition of SOD activity. In addition, loss of mitochondrial membrane potential (MMP), cell cycle arrest, upregulated apoptosis, and enhanced expression of p53 and Jun N-terminal kinase (JNK) were observed in TNP-treated PC12 cells (Wu et al., 2010). Huerta-Garcia et al. (Huerta-Garcia et al., 2014) revealed that TNPs upregulated ROS production in a rat glial cell line (C6) and human glial cell line (U373), accompanied by dysregulated activities of GSH-Px, CAT, and SOD. Wilson et al. (Wilson et al., 2015) also showed that TNPs increased ROS production in rat primary astrocytes, accompanied by loss of MMP, enhanced mitochondrial dynamics-related gene expression, altered mitochondrial morphology, and reduced glutamate uptake, which induced a reduction in cell viability.

Consumers are likely to be exposed to TNPs through dermal exposure. TNP exposure could lead to slight reduction in cell viability of human epidermal cells (A431), which might be attributed to elevated ROS and lipid peroxidation production as well as reduced GSH levels. In addition, genotoxicity, determined by comet assay and micronucleus formation, was observed in TNP-treated A431 cells (Shukla et al., 2011b). Shukla et al. (2011a) showed that TNPs increased ROS production, accompanied by elevated MDA expression, reduced GSH level, and enhanced proportion of apoptotic cells, which contributed to a reduction in cell viability of human keratinocyte cells (HaCaT). Jaeger et al. (Jaeger et al., 2012) discovered that TNPs can upregulate ROS production. Besides, micronucleus formation and mitochondrial DNA damage were observed in TNPs-treated human HaCaT keratinocytes, indicating genotoxicity.

Inhaled TNPs could be deposited in the lung, thereby inducing lung injury. Park et al. (2008) discovered that TNPs increased ROS production and induced GSH depletion in human bronchial epithelial cells (BEAS-2B), accompanied by decreased cell viability and elevated proportion of apoptotic cells. TNPs also reduced cell viability, increased ROS production, and promoted cell apoptosis in

BEAS-2B cells (Shi et al., 2010). Lung mitochondrial dysfunction induced by TNPs was associated with increased ROS production (Freyre-Fonseca et al., 2011). Kansara et al. (2015) further verified that TNPs increased ROS production and inhibited cell cycle in human alveolar cells (A549), accompanied by DNA damage which was determined by elevated micronucleus formation and dysregulated DNA strand breakage-related gene and protein expression.

The liver possesses detoxifying function and could be a target of TNPs as well. ROS production was elevated and DNA damage was observed in TNP-treated human hepatoma cell line (HepG2) (Petkovic et al., 2011). Sha et al. (Sha et al., 2011) revealed that TNP-treated human/rat hepatocellular carcinoma cells and human/rat liver cells exhibited decreased cell viability, upregulated ROS level, and reduced GSH expression. Shukla et al. (2013) also confirmed that TNPs reduced cell viability in HepG2 cells with a concomitant increase in the production of ROS and lipid peroxidation (LPO) as well as a reduction in glutathione level. Besides, DNA damage and apoptosis were observed in TNP-treated HepG2 cells. Besides, upregulated ROS production and inhibited MnSOD activity were observed in TNP-exposed rat primary hepatocytes, accompanied with downregulated expressions of mitochondrial dynamics-related genes and loss of MMP, which might contribute to decreased cell viability and dysfunctional hepatocytes (Natarajan et al., 2015).

Once TNPs are absorbed into circulation, the blood vessel could be affected by TNPs, which might affect the cardiovascular system. Montiel-Davalos et al. (2012) also found that TNPs increased the ROS and NO levels in human umbilical vein endothelial cells (HUVEC) and function of HUVEC was disrupted. Hou et al. (2014) revealed that ROS level in endothelial cells was upregulated by TNP exposure.

Most of absorbed TNPs are excreted from the kidneys. Meena et al. (2012) showed that promoted LDH activity, increased ROS production, elevated MDA level, inhibition of GSH-Px, SOD, and CAT activities, and enhanced proportion of apoptotic cells as well as enhanced comet length were observed in TNP-treated human embryonic kidney cell line (HEK-293), which contributed to the cytotoxicity of TNPs.

Fibroblasts are abound in mammals and they could be affected by TNPs. Jin et al. (2008) displayed that reduced cell viability, elevated levels of ROS and LDH, and decreased GSH expression as well as inhibited SOD activity were observed in TNP-treated mouse fibroblasts. In addition, food grade TNP-treated human lung fibroblasts (WI-38) exhibited decreased cell viability, elevated ROS production, a reduction in mitochondrial membrane potential, and changed cell cycle (Periasamy et al., 2015).

Primary cells or cell lines from other tissues could be impaired by TNP exposure. CAT activity was inhibited, GSH level was reduced, and ROS production increased in TNP-treated human amnion epithelial (WISH) cells, accompanied by decreased cell viability and DNA damage (Saquib et al., 2012). TNPs reduced the viability of human cervical adenocarcinoma HeLa cells, accompanied by elevated levels of MDA, GSSG, and ROS as well as decreased ratio of GSH/GSSG. Meanwhile, Bax expression was promoted with a concomitant reduction in Bcl-2 expression at the gene and protein levels (Ramkumar et al., 2012). TNP-treated rat RBL-2H3 mast cells exhibited upregulated ROS production, with a concomitant increase in histamine secretion as well as  $\text{Ca}^{2+}$  concentration in the cytoplasm (Chen et al., 2012). Decreased cell viability, increased ROS and MDA production, inhibited activities of SOD and CAT, and promoted LDH activity were observed in TNP-treated rat synovial cell line 364, accompanied by cell cycle arrest (Wang et al., 2013a). 8-hydroxy deoxyguanosine (8-oxoDG) level in TNPs-treated human colon carcinoma cells (Caco-2) was promoted (Zijno et al., 2015). TNPs promoted LDH activity, increased ROS and MDA levels, and inhibited the activities of SOD, CAT, and GSH-Px, accom-

panied by loss of MMP and upregulated apoptosis in mouse primary Sertoli cells (Hong et al., 2016).

Findings from *in vitro* and *in vivo* studies are consistent, suggesting that exposure to TNPs could induce OS in primary cells or cell lines from major organs, indicating the toxicity of TNPs. Since OS is implicated in the toxicity of TNPs, how TNPs, the mechanism by which THPs regulated OS should be further discussed.

#### 4. Potential molecular mechanisms by which TNPs regulate oxidative stress

The above-mentioned *in vivo* and *in vitro* studies revealed that OS is involved in the toxicity of TNPs in most cases, but the molecular mechanisms by which TNPs regulate OS are still unclear.

Nuclear factor-E2-related factor-2 (Nrf2) signaling pathway is associated with OS. Wang et al. (2011) found that TNPs could upregulate the levels of  $\text{O}_2^{\bullet-}$ ,  $\text{H}_2\text{O}_2$ , and MDA, with a concomitant elevation in mRNA and protein expression of OS-related genes such as p38, JNK, nuclear factor- $\kappa$ B (NF- $\kappa$ B), Nrf-2, and heme oxygenase-1 (HO-1) in the mouse spleen after intragastric exposure for 30 days. Such TNP-induced OS led to congestion and lymph nodule proliferation in the mouse spleen. Gui et al. (2013) also showed that the TNP content in the mouse kidney was elevated with a concomitant increase in the levels of MDA, protein carbonyl, 8-OHdG,  $\text{O}_2^{\bullet-}$ , and  $\text{H}_2\text{O}_2$ . Meanwhile, the gene and protein expression of Nrf2, HO-1, glutamate cysteine ligase (GCLC), and GST was downregulated accompanied by upregulated levels of Keap-1, NF- $\kappa$ B, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX4), and cyclooxygenase-2 (COX-2). These changes contributed to renal impairments, assessed by histopathological observation and biochemical analysis. When mice received intratracheal administration with TNPs, the NP concentration in the mouse lung increased, accompanied by elevated production of ROS and MDA, which induced lung injury. However, the gene and protein expression of Nrf2, HO-1, and GCLC was upregulated to the highest level at 45 days and then declined (Sun et al., 2012). Ze et al. (2013) displayed that the gene and protein expression of p38, JNK, NF- $\kappa$ B, Nrf2, and HO-1 remained high in the mouse brain after intranasal exposure for 90 days. In addition, the TNP content in the mouse brain was enhanced, accompanied by elevated levels of  $\text{O}_2^{\bullet-}$ ,  $\text{H}_2\text{O}_2$ , MDA, carbonyl, and 8-OHdG.

These studies demonstrated that TNP exposure could regulate Nrf2 expression, thereby inducing OS. Following studies further confirmed Nrf2 regulation in TNP-induced OS. Delgado-Buenrostro et al. (2015) demonstrated that downregulated HO-1 mRNA level and upregulated expression of 3-nitrotyrosine and 4-hydroxynonenal were observed in TNP-treated mice with Nrf2 knockout, when compared to that in exposed wild type mice. Another study showed that oral exposure to TNPs induced higher ROS and MDA levels as well as more severe DNA damage in the liver and kidney of mice with Nrf2 deficiency than that in wildtype mice. *In vitro* experiments revealed that MDA and ARE levels were upregulated, activities of SOD and GSH-Px were inhibited, and gene and protein expression of Nrf2, HO-1, and GCLC was promoted in TNP-treated HepG2 cells. Meanwhile, TNPs caused more severe DNA damage in HepG2 cells with Nrf2 deficiency than in wild type cells (Shi et al., 2015). Findings indicated that Nrf2 acted as a protective factor against TNP-induced OS.

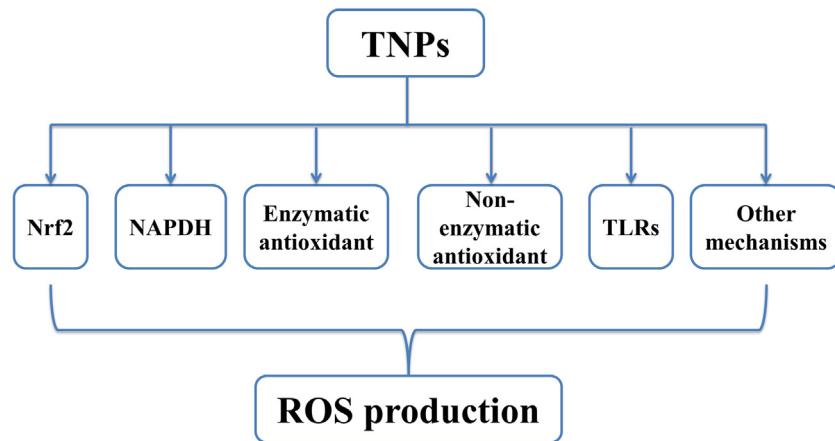
In addition to Nrf2, toll like receptor 4 (TLR4) is correlated with OS. El-Said et al. (2014) revealed that TNPs could increase ROS level and  $\text{H}_2\text{O}_2$  content, inhibit glutathione peroxidase activity, reduce GSH level, and enhance caspase-3 activity in human hepatocarcinoma cells. These changes can be promoted in TNP-treated cells overexpressing TLR4, which suggested that TLR4 might be another factor regulating TNP-induced OS. However, data are far from being

conclusive and TLR4 regulation in TNP-induced OS requires more investigations.

To date, available studies suggest that TNP-induced OS is probably regulated by the Nrf2 signaling pathway. In addition to Nrf2, other molecular mechanisms such as TLR4, by which TNPs regulate OS should be explored further (Fig. 2).

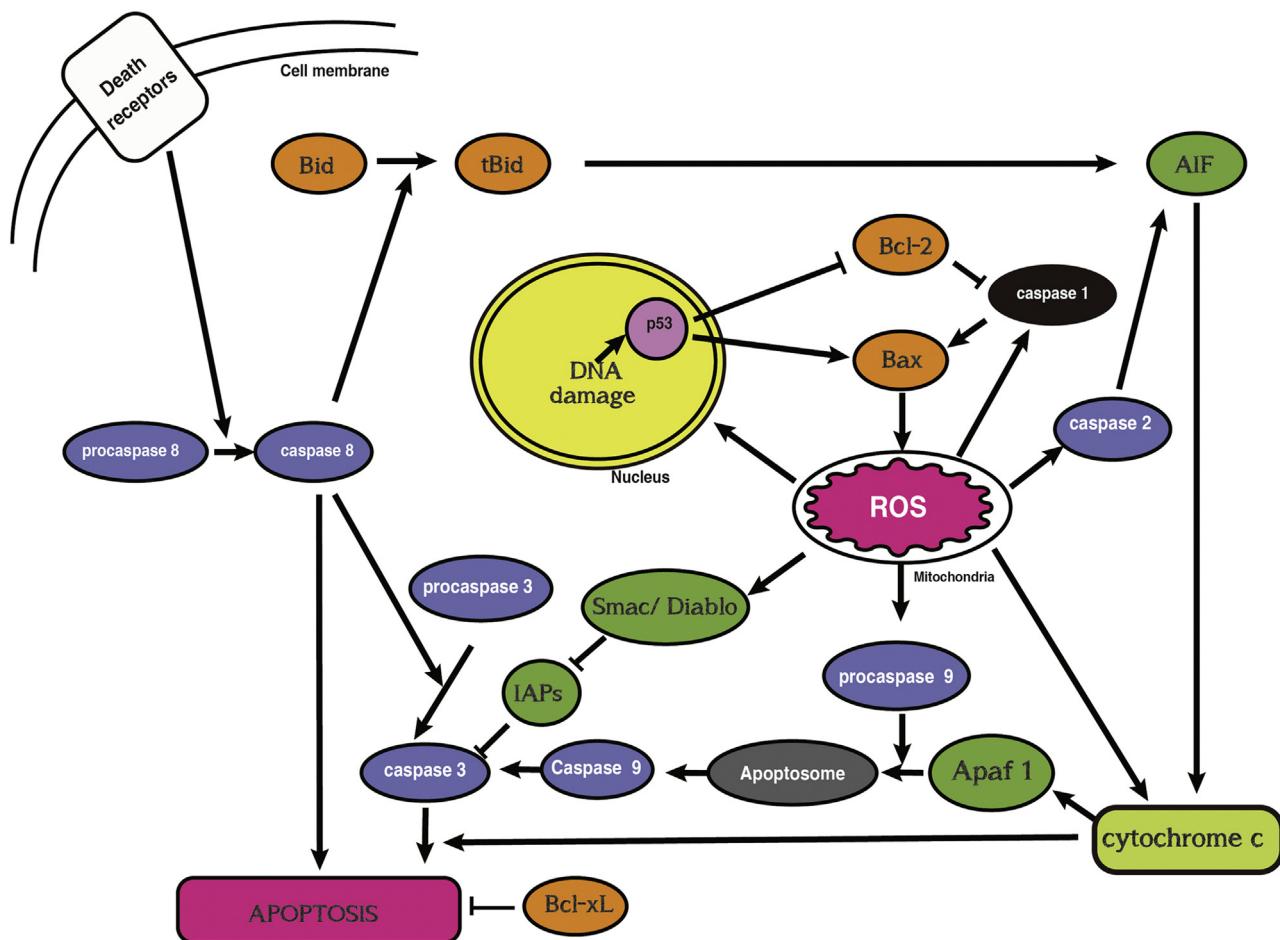
## 5. Cellular responses possibly initiated by TNP-induced OS

*In vivo* and *in vitro* studies revealed that, in addition to OS, apoptosis, inflammatory response, mitochondrial damage, signaling pathways, and genotoxicity were implicated in the toxicity of TNPs. However, whether other cellular responses induced by TNPs were



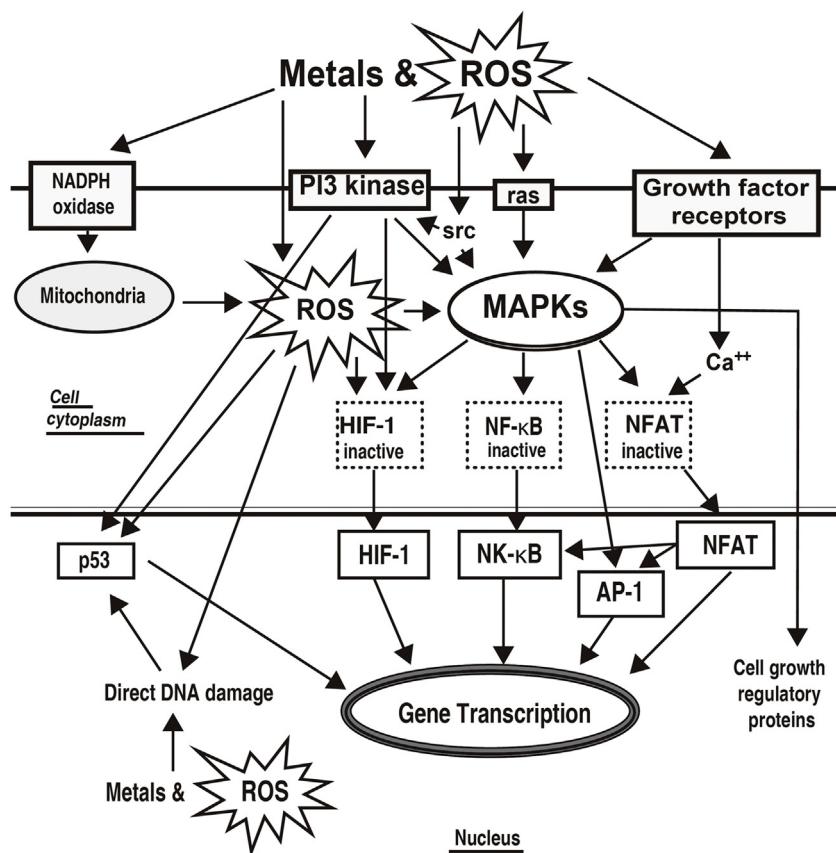
**Fig. 2.** Simple illustration of mechanisms underlying TNPs-induced ROS.

TNPs: titanium dioxide nanoparticles; Nrf2: nuclear factor-E2-related factor-2; NADPH: nicotinamide adenine dinucleotide phosphate; TLRs: toll-like receptors; ROS: reactive oxygen species.



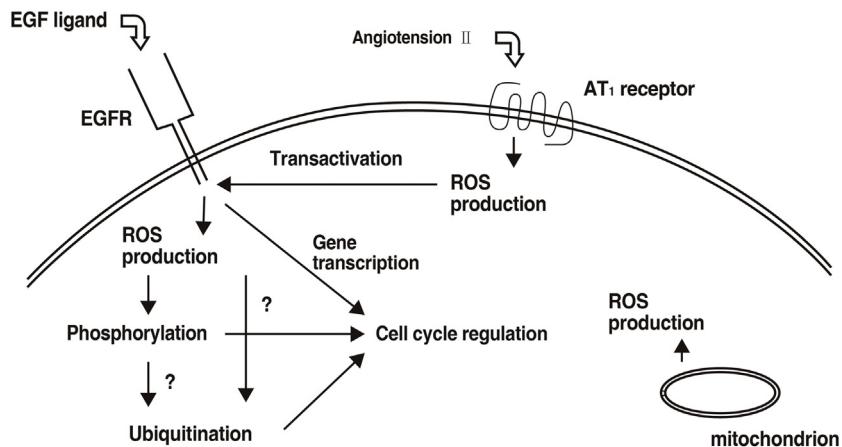
**Fig. 3.** Schematic representation of apoptosis signals induced by ROS (Mates et al., 2008).

AIF: apoptosis-inducing factor; Apaf-1: apoptotic protease activating factor 1; DISC: death inducing signaling complex; ROS: reactive oxygen species; TRAIL: tumor necrosis factor-alpha-related apoptosis-inducing ligand.



**Fig. 4.** Signaling pathways (such as MAPKs) activated by ROS (Leonard et al., 2004).

ROS: reactive oxygen species; NADPH: reduced nicotine adenine dinucleotide phosphate; MAPK: mitogen-activated protein kinase; HIF-1: hypoxia-inducible factor 1; NF-κB: necrosis factor kappa B; NFAT: nuclear factor of activated T cells; AP-1:activator protein-1.



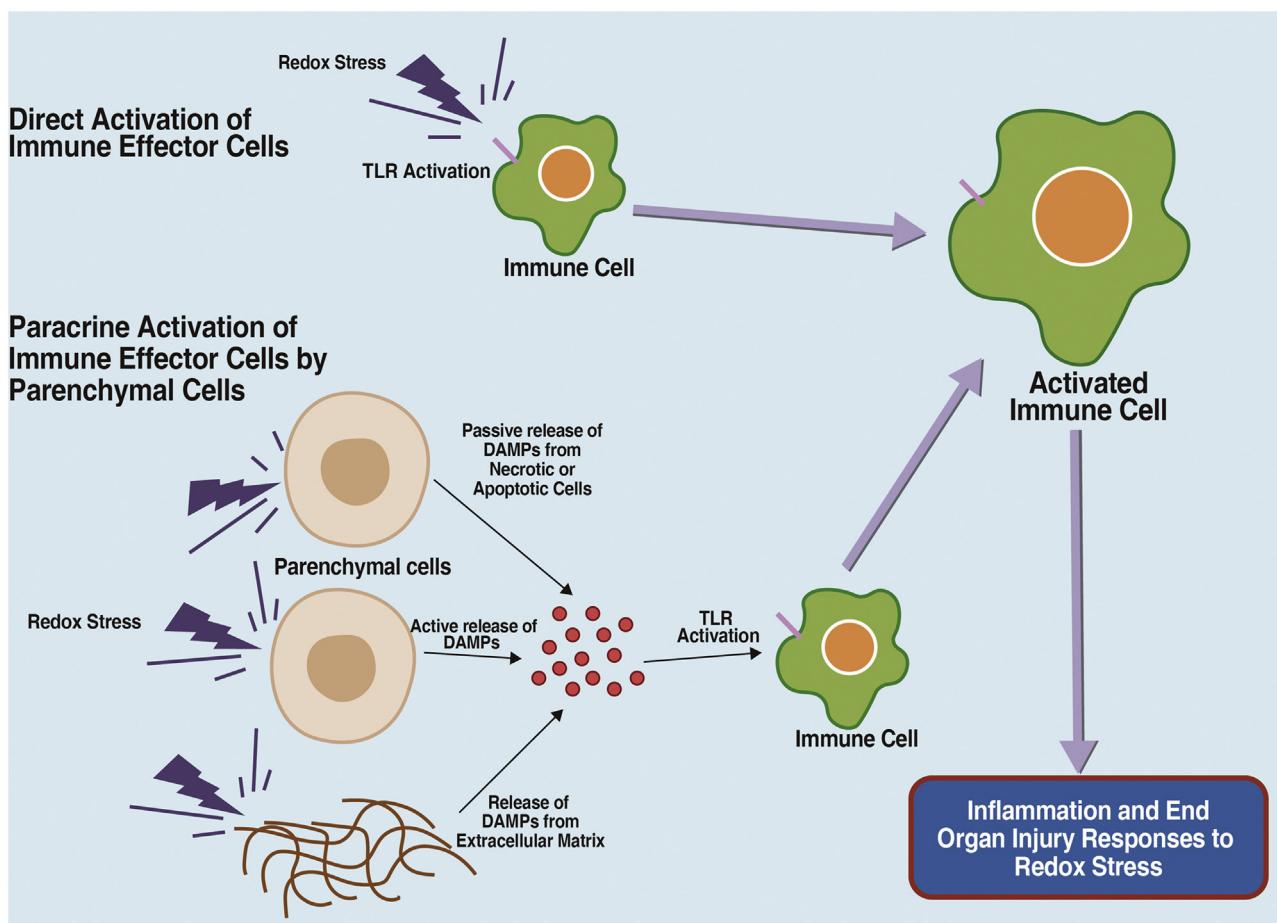
**Fig. 5.** The effects of ROS on cell cycle regulation (Verbon et al., 2012).

EGFR: epidermal growth factor receptor; EGF: epidermal growth factor; ROS: reactive oxygen species.

initiated by OS remains unclear. Given the pivotal of OS in cellular responses such as apoptosis (Fig. 3), signaling pathways (Fig. 4), cell cycle (Fig. 5), and inflammatory response (Fig. 6), we hypothesized that the toxicity of TNPs might be the cascades of TNP-induced OS. To date, a few rescue studies shed some light on this obscure issue and verified our hypothesis to a certain extent.

### 5.1. In vivo studies

Orazizadeh et al. (2014) revealed that gavage administration of TNPs increased the rat blood levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP). Meanwhile, MDA level was upregulated and the activities of



**Fig. 6.** Inflammatory response through TLRs activation mediated by OS (Gill et al., 2010).  
DAMPs: damage-associated molecular pattern molecules; TLR: toll-like receptor.

GSH-Px and SOD were inhibited in TNP-treated rat liver, accompanied by increased proportion of apoptotic cells. However, these toxic effects were abrogated by pretreatment with glycyrrhizic acid before TNP administration. Azim et al. (2015) found that oral exposure to TNPs led to enhanced MDA level and reduced GSH expression in mouse livers. Meanwhile, Nrf2 and NF- $\kappa$ B mRNA expression was elevated, accompanied by an increased proportion of CD68 $^{+}$  cells as well as increased levels of tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and interleukin-6 (IL-6). In addition, the caspase-3 activity was promoted and Bax gene expression was elevated, accompanied by decreased Bcl-2 expression. Moreover, DNA damage was observed in the TNP-treated group. However, these toxic effects on mouse livers were attenuated by oral treatment with idebenone (ID), carnosine (CR), vitamin E (Vit. E), or the combination of ID, CR, and Vit.E for one month following TNP exposure. TNPs induced a greater OS to the kidney than to the liver after rats were exposed, which was attenuated by quercetin treatment (Gonzalez-Esquivel et al., 2015).

It is accepted that Vit. E (Traber and Atkinson, 2007), ID (Fetoni et al., 2008), CR (Aldini et al., 2011), glycyrrhizic acid (Ming and Yin, 2013), and quercetin (Costa et al., 2016) act as anti-oxidants. Findings indicated that the toxic effects induced by TNPs could be attenuated through blocking TNP-induced OS by anti-oxidants.

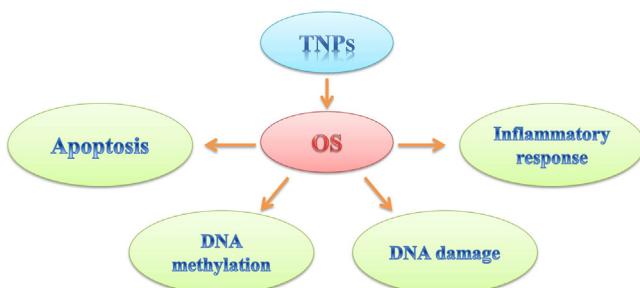
## 5.2. In vitro studies

Hussain et al. (2009) showed that TNPs can be taken up by human bronchial epithelial cells (16HBE14o-) and increase

ROS production. Moreover, granulocyte-macrophage colony-stimulating factor (GM-CSF) gene level in 16HBE14o- cells was elevated, which was abrogated by co-treatment with CAT. Niska et al. (2015) also found that TNPs can reduce the cell viability of human osteoblast cells (Hfob 1.19). Moreover, O<sub>2</sub> $^{•-}$  production increased, protein levels of CuZnSOD (SOD1), MnSOD (SOD2), and sirtuin 3 (SIR3) decreased, SOD and CAT activities were inhibited, and MDA level was elevated. These changes were abrogated by pretreatment with SOD.

TNPs upregulated ROS production in BEAS-2B cells, which was attenuated by co-treatment with desferal (Bhattacharya et al., 2009). TNPs could increase ROS production and the percentage of apoptotic cells, which contributed to reduced cell viability in PC12 cells. These effects were abrogated by pretreatment with N-(mercaptopropionyl)-glycine (N-MPG) (Liu et al., 2010). Aueviriyavit et al. found that ROS production in TNPs-treated A549 cells was elevated (Aueviriyavit et al., 2012). Han et al. (2013) confirmed that the levels of vascular cell adhesion molecule-1 (VCAM-1) and monocyte chemoattractant protein-1 (MCP-1) were enhanced in TNP-treated primary vascular endothelial cells, which was abrogated by pretreatment with epigallocatechin gallate and apocynin. Another study showed that the methylation level of poly (ADP-ribose) polymerase 1 (PARP-1) was upregulated in TNP-treated A549 cells, which was attenuated by co-treatment with  $\alpha$ -lipoic acid (Bai et al., 2015).

CAT and SOD are common enzymatic antioxidants. Meanwhile, desferal (Yousdim et al., 2004), epigallocatechin gallate (Na et al., 2008), apocynin (Heumuller et al., 2008), N-MPG (Fantinelli et al.,



**Fig. 7.** Cellular responses initiated by TNPs-induced OS.  
TNPs: titanium dioxide nanoparticles; OS: oxidative stress.

2013), and  $\alpha$ -lipoic acid (Rochette et al., 2013) also function as antioxidants. These *in vitro* studies suggested that abrogating TNP-induced OS by these substances alleviated the toxicity of TNPs in primary cells or cell lines.

These rescue studies demonstrated that blocking TNP-induced OS by antioxidants could abrogate cellular responses such as apoptosis, inflammatory response, DNA methylation, and DNA damage that contributed to the toxicity of TNPs (Fig. 7). However, findings are far from being conclusive and more rescue studies are warranted to further investigate whether cellular responses were initiated by TNP-induced OS. Unraveling the role of OS in the toxicity of TNPs might shed some light on methods to improve the bio-safety of TNP-enabled products (Pan et al., 2009).

## 6. Conclusion

In summary, widespread applications of TNP-enabled products increase the risk of unintentional exposure, which can, in turn, induce toxic effects. Given the critical role of OS, we summarized relevant articles covering the involvement of OS in TNPs' toxicity. Subsequently, we found that OS was implicated in the toxicity of TNPs in most situations. However, data are far from being conclusive and more studies are required to comprehensively investigate the molecular mechanisms by which TNPs regulate OS. Moreover, whether the toxicity of TNPs is mediated by signaling cascades involved in OS should be fully investigated. Unraveling the role of OS in TNPs' toxicity might help us to comprehensively understanding the correlation between TNP-induced cellular responses, and could shed some light on methods to alleviate the toxic effects of TNPs.

## Competing interests

The authors declare that they have no competing interests.

## Acknowledgements

This work was supported by the Science and Technology Joint Foundation of Guizhou province, China (QKH-LHZ(2016)7159), the Science and Technology Joint Foundation of Guizhou province, China (QKH-LHZ(2016)7160), the National Natural Science Foundation of China (81550011), and the Natural Science Foundation of Guangdong Province of China (2015A030313299).

## References

- Ahmadi, A., Shadboorestan, A., 2016. Oxidative stress and cancer; the role of hesperidin, a citrus natural bioflavonoid, as a cancer chemoprotective agent. *Nutr. Cancer* 68, 29–39.
- Aldini, G., Orioli, M., Rossoni, G., Savi, F., Braidotti, P., Vistoli, G., Yeum, K.J., Negrisoli, G., Carini, M., 2011. The carbonyl scavenger carnosine ameliorates dyslipidaemia and renal function in Zucker obese rats. *J. Cell. Mol. Med.* 15, 1339–1354.
- Aueviriyavit, S., Phummiratch, D., Kulthong, K., Maniratanachote, R., 2012. Titanium dioxide nanoparticles-mediated *in vitro* cytotoxicity does not induce Hsp70 and Grp78 expression in human bronchial epithelial A549 cells. *Biol. Trace Elem. Res.* 149, 123–132.
- Azim, S.A.A., Darwish, H.A., Rizk, M.Z., Ali, S.A., Kadry, M.O., 2015. Amelioration of titanium dioxide nanoparticles-induced liver injury in mice: possible role of some antioxidants. *Exp. Toxicol. Pathol.* 67, 305–314.
- Bai, W.L., Chen, Y.J., Gao, A., 2015. Cross talk between poly(ADP-ribose) polymerase 1 methylation and oxidative stress involved in the toxic effect of anatase titanium dioxide nanoparticles. *Int. J. Nanomed.* 10, 5561–5569.
- Bhattacharya, K., Davoren, M., Boertz, J., Schins, R.P.F., Hoffmann, E., Dopp, E., 2009. Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells. *Part. Fibre Toxicol.* 6, 11.
- Bonomini, F., Rodella, L.F., Rezzani, R., 2015. Metabolic syndrome, aging and involvement of oxidative stress. *Aging Dis.* 6, 109–120.
- Chen, X., Mao, S.S., 2007. Titanium dioxide nanomaterials Synthesis, properties, modifications, and applications. *Chem. Rev.* 107, 2891–2959.
- Chen, J.Y., Dong, X., Zhao, J., Tang, G.P., 2009. *In vivo* acute toxicity of titanium dioxide nanoparticles to mice after intraperitoneal injection. *J. Appl. Toxicol.* 29, 330–337.
- Chen, E.Y., Garnica, M., Wang, Y.C., Mintz, A.J., Chen, C.S., Chin, W.C., 2012. A mixture of anatase and rutile TiO<sub>2</sub> nanoparticles induces histamine secretion in mast cells. *Part. Fibre Toxicol.* 9, 10.
- Clemente, Z., Castro, V.L., Feitosa, L.O., Lima, R., Jonsson, C.M., Maia, A.H.N., Fraceto, L.F., 2015. Biomarker evaluation in fish after prolonged exposure to nano-TiO<sub>2</sub>: influence of illumination conditions and crystal phase. *J. Nanosci. Nanotechnol.* 15, 5424–5433.
- Costa, L.G., Garrick, J.M., Roque, P.J., Pellacani, C., 2016. Mechanisms of neuroprotection by quercetin: counteracting oxidative stress and more. *Oxid. Med. Cell. Longevity.*
- Cui, Y.L., Gong, X.L., Duan, Y.M., Li, N., Hu, R.P., Liu, H.T., Hong, M.M., Zhou, M., Wang, L., Wang, H., Hong, F.S., 2010. Hepatocyte apoptosis and its molecular mechanisms in mice caused by titanium dioxide nanoparticles. *J. Hazard. Mater.* 183, 874–880.
- Cui, Y.L., Liu, H.T., Ze, Y.G., Zhang, Z.L., Hu, Y.Y., Cheng, Z., Cheng, J., Hu, R.P., Gao, G.D., Wang, L., Tang, M., Hong, F.S., 2012. Gene expression in liver injury caused by long-term exposure to titanium dioxide nanoparticles in mice. *Toxicol. Sci.* 128, 171–185.
- da Rosa, E.L.S., 2013. Kinetic effects of TiO<sub>2</sub> fine particles and nanoparticles aggregates on the nanomechanical properties of human neutrophils assessed by force spectroscopy. *BMC Biophys.* 6.
- Delgado-Buenrostro, N.L., Medina-Reyes, E.I., Lastres-Becker, I., Freyre-Fonseca, V., Ji, Z.X., Hernandez-Pando, R., Marquina, B., Pedraza-Chaverri, J., Espada, S., Cuadrado, A., Chirino, Y.I., 2015. Nrf2 protects the lung against inflammation induced by titanium dioxide nanoparticles: a positive regulator role of Nrf2 on cytokine release. *Environ. Toxicol.* 30, 782–792.
- Deng, D., Kim, M.G., Lee, J.Y., Cho, J., 2009. Green energy storage materials: nanostructured TiO<sub>2</sub> and Sn-based anodes for lithium-ion batteries. *Energy Environ. Sci.* 2, 818–837.
- El-Said, K.S., Ali, E.M., Kanehira, K., Taniguchi, A., 2014. Molecular mechanism of DNA damage induced by titanium dioxide nanoparticles in toll-like receptor 3 or 4 expressing human hepatocarcinoma cell lines. *J. Nanobiotechnology* 12, 10.
- Elgrably, D., Beaudouin, R., Jbilou, N., Floriani, M., Pery, A., Rogerieux, F., Lacroix, G., 2015. Biodistribution and clearance of TiO<sub>2</sub> nanoparticles in rats after intravenous injection. *PLoS One* 10.
- Fabian, E., Landsiedel, R., Ma-Hock, L., Wiench, K., Wohlleben, W., van Ravenzwaay, B., 2008. Tissue distribution and toxicity of intravenously administered titanium dioxide nanoparticles in rats. *Arch. Toxicol.* 82, 151–157.
- Fantinelli, J.C., Arbelaez, L.F.G., Nunez, I.A.P., Mosca, S.M., 2013. Protective effects of N-(2-mercaptopropionyl)-glycine against ischemia-reperfusion injury in hypertrophied hearts. *Exp. Mol. Pathol.* 94, 277–284.
- Fetoni, A.R., Ferraresi, A., La Greca, C., Rizzo, D., Sergi, B., Tringali, G., Piacentini, R., Troiani, D., 2008. Antioxidant protection against acoustic trauma by coadministration of idebenone and vitamin E. *Neuroreport* 19, 277–281.
- Freyre-Fonseca, V., Delgado-Buenrostro, N.L., Gutierrez-Cirlos, E.B., Calderon-Torres, C.M., Cabellos-Avelar, T., Sanchez-Perez, Y., Pinzon, E., Torres, I., Molina-Jijon, E., Zazueta, C., Pedraza-Chaverri, J., Garcia-Cuellar, C.M., Chirino, Y.I., 2011. Titanium dioxide nanoparticles impair lung mitochondrial function. *Toxicol. Lett.* 202, 111–119.
- Gao, G.D., Ze, Y.G., Li, B., Zhao, X.Y., Zhang, T., Sheng, L., Hu, R.H., Gui, S.X., Sang, X.Z., Sun, Q.Q., Cheng, J., Cheng, Z., Wang, L., Tang, M., Hong, F.H., 2012. Ovarian dysfunction and gene-expressed characteristics of female mice caused by long-term exposure to titanium dioxide nanoparticles. *J. Hazard. Mater.* 243, 19–27.
- Geraets, L., Oomen, A.G., Krystek, P., Jacobsen, N.R., Wallin, H., Laurentie, M., Verharen, H.W., Brandon, E.F.A., de Jong, W.H., 2014. Tissue distribution and elimination after oral and intravenous administration of different titanium dioxide nanoparticles in rats. *Part. Fibre Toxicol.* 11.
- Gheshlaghi, Z.N., Riazi, G.H., Ahmadian, S., Ghafari, M., Mahinpour, R., 2008. Toxicity and interaction of titanium dioxide nanoparticles with microtubule protein. *Acta Biochim. Biophys. Sin.* 40, 777–782.
- Gill, R., Tsung, A., Billiar, T., 2010. Linking oxidative stress to inflammation: toll-like receptors. *Free Radic. Biol. Med.* 48, 1121–1132.
- Giovanni, M., Yue, J.Q., Zhang, L.F., Xie, J.P., Ong, C.N., Leong, D.T., 2015. Pro-inflammatory responses of RAW264.7 macrophages when treated with

- ultralow concentrations of silver titanium dioxide, and zinc oxide nanoparticles. *J. Hazard. Mater.* 297, 146–152.
- Gitrowski, C., Al-Jubury, A.R., Handy, R.D., 2014. Uptake of different crystal structures of TiO<sub>2</sub> nanoparticles by Caco-2 intestinal cells. *Toxicol. Lett.* 226, 264–276.
- Gonzalez-Esquivel, A.E., Charles-Nino, C.L., Pacheco-Moises, F.P., Ortiz, G.G., Jaramillo-Juarez, F., Rincon-Sanchez, A.R., 2015. Beneficial effects of quercetin on oxidative stress in liver and kidney induced by titanium dioxide (TiO<sub>2</sub>) nanoparticles in rats. *Toxicol. Mech. Methods* 25, 166–175.
- Gui, S.X., Li, B.Y., Zhao, X.Y., Sheng, L., Hong, J., Yu, X.H., Sang, X.Z., Sun, Q.Q., Ze, Y.G., Wang, L., Hong, F.S., 2013. Renal injury and nrf2 modulation in mouse kidney following chronic exposure to TiO<sub>2</sub> nanoparticles. *J. Agric. Food Chem.* 61, 8959–8968.
- Han, S.G., Newsome, B., Hennig, B., 2013. Titanium dioxide nanoparticles increase inflammatory responses in vascular endothelial cells. *Toxicology* 306, 1–8.
- Hendrickson, O.D., Pridvorova, S.M., Zherdev, A.V., Klochkov, S.G., Novikova, O.V., Shevtsova, E.F., Bachurin, S.O., Dzantiev, B.B., 2016. Size-dependent differences in biodistribution of titanium dioxide nanoparticles after sub-acute intragastric administrations to rats. *Curr. Nanosci.* 12, 228–236.
- Heumuller, S., Wind, S., Barbosa-Sicard, E., Schmidt, H., Busse, R., Schroder, K., Brandes, R.P., 2008. Apocynin is not an inhibitor of vascular NADPH oxidases but an antioxidant. *Hypertension* 51, 211–217.
- Hong, F.S., Zhao, X.Y., Chen, M., Zhou, Y.J., Ze, Y.G., Wang, L., Wang, Y.J., Ge, Y.S., Zhang, Q., Ye, L.Q., 2016. TiO<sub>2</sub> nanoparticles-induced apoptosis of primary cultured Sertoli cells of mice. *J. Biomed. Mater. Res. Part A* 104, 124–135.
- Hou, Y.H., Lai, M., Chen, X.Y., Li, J.H., Hu, Y., Luo, Z., Ding, X.W., Cai, K.Y., 2014. Effects of mesoporous SiO<sub>2</sub>, Fe3O4, and TiO<sub>2</sub> nanoparticles on the biological functions of endothelial cells in vitro. *J. Biomed. Mater. Res. Part A* 102, 1726–1736.
- Hsiao, I.L., Chang, C.C., Wu, C.Y., Hsieh, Y.K., Chuang, C.Y., Wang, C.F., Huang, Y.J., 2016. Indirect effects of TiO<sub>2</sub> nanoparticle on neuron-glia cell interactions. *Chem.-Biol. Interact.* 254, 34–44.
- Hsu, L.Y., Chein, H.M., 2007. Evaluation of nanoparticle emission for TiO<sub>2</sub> nanopowder coating materials. *J. Nanopart. Res.* 9, 157–163.
- Hu, R., Zheng, L., Zhang, T., Gao, G., Cui, Y., Cheng, Z., Cheng, J., Hong, M., Tang, M., Hong, F., 2011. Molecular mechanism of hippocampal apoptosis of mice following exposure to titanium dioxide nanoparticles. *J. Hazard. Mater.* 191, 32–40.
- Hu, H.L., Guo, Q., Wang, C.L., Ma, X., He, H.J., Oh, Y.R., Feng, Y.J., Wu, Q., Gu, N., 2015. Titanium dioxide nanoparticles increase plasma glucose via reactive oxygen species-induced insulin resistance in mice. *J. Appl. Toxicol.* 35, 1122–1132.
- Huerta-Garcia, E., Perez-Arizti, J.A., Marquez-Ramirez, S.G., Delgado-Buenrostro, N.L., Chirino, Y.I., Iglesias, G.G., Lopez-Marure, R., 2014. Titanium dioxide nanoparticles induce strong oxidative stress and mitochondrial damage in glial cells. *Free Radic. Biol. Med.* 73, 84–94.
- Huerta-Garcia, E., Marquez-Ramirez, S.G., Ramos-Godinez, M.D., Lopez-Saavedra, A., Herrera, L.A., Parra, A., Alfaro-Moreno, E., Gomez, E.O., Lopez-Marure, R., 2015. Internalization of titanium dioxide nanoparticles by glial cells is given at short times and is mainly mediated by actin reorganization-dependent endocytosis. *Neurotoxicology* 51, 27–37.
- Hussain, S., Boland, S., Baeza-Squiban, A., Hamel, R., Thomassen, L.C.J., Martens, J.A., Billon-Galland, M.A., Fleury-Feith, J., Moisan, F., Paireon, J.C., Marano, F., 2009. Oxidative stress and proinflammatory effects of carbon black and titanium dioxide nanoparticles: role of particle surface area and internalized amount. *Toxicology* 260, 142–149.
- Jaeger, A., Weiss, D.G., Jonas, L., Kriehuber, R., 2012. Oxidative stress-induced cytotoxic and genotoxic effects of nano-sized titanium dioxide particles in human HaCaT keratinocytes. *Toxicology* 296, 27–36.
- Jin, C.Y., Zhu, B.S., Wang, X.F., Lu, Q.H., 2008. Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. *Chem. Res. Toxicol.* 21, 1871–1877.
- Jones, D.P., 2006. Redefining oxidative stress. *Antioxid. Redox Signal.* 8, 1865–1879.
- Kansara, K., Patel, P., Shah, D., Shukla, R.K., Singh, S., Kumar, A., Dhawan, A., 2015. TiO<sub>2</sub> nanoparticles induce DNA double strand breaks and cell cycle arrest in human alveolar cells. *Environ. Mol. Mutagen.* 56, 204–217.
- Leonard, S.S., Harris, G.K., Shi, X.L., 2004. Metal-induced oxidative stress and signal transduction. *Free Radic. Biol. Med.* 37, 1921–1942.
- Li, Q.L., Mahendra, S., Lyon, D.Y., Brunet, L., Liga, M.V., Li, D., Alvarez, P.J.J., 2008. Antimicrobial nanomaterials for water disinfection and microbial control: potential applications and implications. *Water Res.* 42, 4591–4602.
- Liang, G.Y., Pu, Y.P., Yin, L.H., Liu, R., Ye, B., Su, Y.Y., Li, Y.F., 2009. Influence of different sizes of titanium dioxide nanoparticles on hepatic and renal functions in rats with correlation to oxidative stress. *J. Toxicol. Env. Health Part A* 72, 740–745.
- Liu, H.T., Ma, L.L., Zhao, J.F., Liu, J., Yan, J.Y., Ruan, J., Hong, F.S., 2009. Biochemical toxicity of nano-anatase TiO<sub>2</sub> particles in mice. *Biol. Trace Elem. Res.* 129, 170–180.
- Liu, S.C., Xu, L.J., Zhang, T., Ren, G.G., Yang, Z., 2010. Oxidative stress and apoptosis induced by nanosized titanium dioxide in PC12 cells. *Toxicology* 267, 172–177.
- Liu, H.L., Yang, D.F., Yang, H.L., Zhang, H.S., Zhang, W., Fang, Y.J., Lin, Z.Q., Tian, L., Lin, B.C., Yan, J., Xi, Z.G., 2013. Comparative study of respiratory tract immune toxicity induced by three sterilisation nanoparticles: silver, zinc oxide and titanium dioxide. *J. Hazard. Mater.* 248, 478–486.
- Lopes, V.R., Loitto, V., Audinot, J.N., Bayat, N., Gutleb, A.C., Cristobal, S., 2016. Dose-dependent autophagic effect of titanium dioxide nanoparticles in human HaCaT cells at non-cytotoxic levels. *J. Nanobiotechnology* 14.
- Lu, P.J., Huang, S.C., Chen, Y.P., Chiueh, L.C., Shih, D.Y.C., 2015. Analysis of titanium dioxide and zinc oxide nanoparticles in cosmetics. *J. Food Drug Anal.* 23, 587–594.
- Ma, L.L., Liu, J., Li, N., Wang, J., Duan, Y.M., Yan, J.Y., Liu, H.T., Wang, H., Hong, F.S., 2010. Oxidative stress in the brain of mice caused by translocated nanoparticulate TiO<sub>2</sub> delivered to the abdominal cavity. *Biomaterials* 31, 99–105.
- Mao, Z.L., Xu, B., Ji, X.L., Zhou, K., Zhang, X.M., Chen, M.J., Han, X.M., Tang, Q.S., Wang, X.R., Xia, Y.K., 2015. Titanium dioxide nanoparticles alter cellular morphology via disturbing the microtubule dynamics. *Nanoscale* 7, 8466–8475.
- Marquez-Ramirez, S.G., Delgado-Buenrostro, N.L., Chirino, Y.I., Iglesias, G.G., Lopez-Marure, R., 2012. Titanium dioxide nanoparticles inhibit proliferation and induce morphological changes and apoptosis in glial cells. *Toxicology* 302, 146–156.
- Mates, J.M., Segura, J.A., Alonso, F.J., Marquez, J., 2008. Intracellular redox status and oxidative stress: implications for cell proliferation apoptosis, and carcinogenesis. *Arch. Toxicol.* 82, 273–299.
- Meena, R., Rani, M., Pal, R., Rajamani, P., 2012. Nano-TiO<sub>2</sub>-induced apoptosis by oxidative stress-mediated DNA damage and activation of p53 in human embryonic kidney cells. *Appl. Biochem. Biotechnol.* 167, 791–808.
- Meena, R., Kajal, K., Paulraj, R., 2015a. Cytotoxic and genotoxic effects of titanium dioxide nanoparticles in testicular cells of male wistar rat. *Appl. Biochem. Biotechnol.* 175, 825–840.
- Meena, R., Kumar, S., Paulraj, R., 2015b. Titanium oxide (TiO<sub>2</sub>) nanoparticles in induction of apoptosis and inflammatory response in brain. *J. Nanopart. Res.* 17.
- Ming, L.J., Yin, A.C.Y., 2013. Therapeutic effects of glycyrrhizic acid. *Nat. Prod. Commun.* 8, 415–418.
- Miricescu, D., Totan, A., Calenic, B., Mocanu, B., Didilescu, A., Mohora, M., Spinu, T., Greabu, M., 2014. Salivary biomarkers: relationship between oxidative stress and alveolar bone loss in chronic periodontitis. *Acta Odontol. Scand.* 72, 42–47.
- Mohammadipour, A., Fazel, A., Haghiri, H., Motejaded, F., Rafatpanah, H., Zabibi, H., Hosseini, M., Bideskan, A.E., 2014. Maternal exposure to titanium dioxide nanoparticles during pregnancy; impaired memory and decreased hippocampal cell proliferation in rat offspring. *Environ. Toxicol. Pharmacol.* 37, 617–625.
- Mohammadipour, A., Hosseini, M., Fazel, A., Haghiri, H., Rafatpanah, H., Pourganji, M., Bideskan, A.E., 2016. The effects of exposure to titanium dioxide nanoparticles during lactation period on learning and memory of rat offspring. *Toxicol. Ind. Health* 32, 221–228.
- Montazer, M., Seifollahzadeh, S., 2011. Enhanced self-cleaning, antibacterial and UV protection properties of nano TiO<sub>2</sub> treated textile through enzymatic pretreatment. *Photochem. Photobiol.* 87, 877–883.
- Montiel-Davalos, A., Ventura-Gallegos, J.L., Alfaro-Moreno, E., Soria-Castro, E., Garcia-Latorre, E., Cabanas-Moreno, J.G., Ramos-Godinez, M.D., Lopez-Marure, R., 2012. TiO<sub>2</sub> nanoparticles induce dysfunction and activation of human endothelial cells. *Chem. Res. Toxicol.* 25, 920–930.
- Na, H.K., Kim, E.H., Jung, J.H., Lee, H.H., Hyun, J.W., Surh, Y.J., 2008. (−)-epigallocatechin gallate induces Nrf2-mediated antioxidant enzyme expression via activation of PI3K and ERK in human mammary epithelial cells. *Arch. Biochem. Biophys.* 476, 171–177.
- Natarajan, V., Wilson, C.L., Hayward, S.L., Kidambi, S., 2015. Titanium dioxide nanoparticles trigger loss of function and perturbation of mitochondrial dynamics in primary hepatocytes. *PLoS One* 10, 19.
- Neacsu, P., Mazare, A., Schmuki, P., Cimpean, A., 2015. Attenuation of the macrophage inflammatory activity by TiO<sub>2</sub> nanotubes via inhibition of MAPK and NF-κappa B pathways. *Int. J. Nanomed.* 10, 6455–6467.
- Niska, K., Pyszka, K., Tukaj, C., Woźniak, M., Radomski, M.W., Inkielewicz-Stepniak, I., 2015. Titanium dioxide nanoparticles enhance production of superoxide anion and alter the antioxidant system in human osteoblast cells. *Int. J. Nanomed.* 10, 1095–1107.
- Orazizadeh, M., Fakhredini, F., Mansouri, E., Khorsandi, L., 2014. Effect of glycyrrhizic acid on titanium dioxide nanoparticles-induced hepatotoxicity in rats. *Chem.-Biol. Interact.* 220, 214–221.
- Pan, Z., Lee, W., Slutsky, L., Clark, R.A.F., Pernodet, N., Rafailovich, M.H., 2009. Adverse effects of titanium dioxide nanoparticles on human dermal fibroblasts and how to protect cells. *Small* 5, 511–520.
- Park, E.J., Yi, J., Chung, Y.H., Ryu, D.Y., Choi, J., Park, K., 2008. Oxidative stress and apoptosis induced by titanium dioxide nanoparticles in cultured BEAS-2B cells. *Toxicol. Lett.* 180, 222–229.
- Patri, A., Umbreit, T., Zheng, J., Nagashima, K., Goering, P., Francke-Carroll, S., Gordon, E., Weaver, J., Miller, T., Sadrieh, N., McNeil, S., Stratmeyer, M., 2009. Energy dispersive X-ray analysis of titanium dioxide nanoparticle distribution after intravenous and subcutaneous injection in mice. *J. Appl. Toxicol.* 29, 662–672.
- Pelclova, D., Zdimal, V., Fenclova, Z., Vlckova, S., Turci, F., Corazzari, I., Kacer, P., Schwarz, J., Zikova, N., Makes, O., Syslova, K., Komarc, M., Belacek, J., Navratil, T., Machajova, M., Zakharov, S., 2016. Markers of oxidative damage of nucleic acids and proteins among workers exposed to TiO<sub>2</sub> (nano) particles. *Occup. Environ. Med.* 73, 110–118.
- Periasamy, V.S., Athinarakayanan, J., Al-Hadi, A.M., Al Juhaimi, F., Mahmoud, M.H., Alshatwi, A.A., 2015. Identification of titanium dioxide nanoparticles in food products: induce intracellular oxidative stress mediated by TNF and CYP1A genes in human lung fibroblast cells. *Environ. Toxicol. Pharmacol.* 39, 176–186.
- Petkovic, J., Zegura, B., Stevanovic, M., Drnovsek, N., Uskokovic, D., Novak, S., Filipic, M., 2011. DNA damage and alterations in expression of DNA damage

- responsive genes induced by TiO<sub>2</sub> nanoparticles in human hepatoma HepG2 cells. *Nanotoxicology* 5, 341–353.
- Ramkumar, K.M., Manjula, C., GnanaKumar, G., Kanjwal, M.A., Sekar, T.V., Paulmurugan, R., Rajaguru, P., 2012. Oxidative stress-mediated cytotoxicity and apoptosis induction by TiO<sub>2</sub> nanofibers in HeLa cells. *Eur. J. Pharm. Biopharm.* 81, 324–333.
- Rochette, L., Ghibu, S., Richard, C., Zeller, M., Cottin, Y., Vergely, C., 2013. Direct and indirect antioxidant properties of alpha-lipoic acid and therapeutic potential. *Mol. Nutr. Food Res.* 57, 114–125.
- Sang, L.X., Zhao, Y.X., Burda, C., 2014. TiO<sub>2</sub> nanoparticles as functional building blocks. *Chem. Rev.* 114, 9283–9318.
- Saqib, Q., Al-Khedhairy, A.A., Siddiqui, M.A., Abou-Tarboush, F.M., Azam, A., Musarrat, J., 2012. Titanium dioxide nanoparticles induced cytotoxicity, oxidative stress and DNA damage in human amnion epithelial (WISH) cells. *Toxicol. Vitro* 26, 351–361.
- Sha, B.Y., Gao, W., Wang, S.Q., Xu, F., Lu, T.J., 2011. Cytotoxicity of titanium dioxide nanoparticles differs in four liver cells from human and rat. *Compos. Part. B: Eng.* 42, 2136–2144.
- Shi, Y.L., Wang, F., He, J.B., Yadav, S., Wang, H., 2010. Titanium dioxide nanoparticles cause apoptosis in BEAS-2B cells through the caspase 8/t-Bid-independent mitochondrial pathway. *Toxicol. Lett.* 196, 21–27.
- Shi, Z.Q., Niu, Y.J., Wang, Q., Shi, L., Guo, H.C., Liu, Y., Zhu, Y., Liu, S.F., Liu, C., Chen, X., Zhang, R., 2015. Reduction of DNA damage induced by titanium dioxide nanoparticles through Nrf2 in vitro and in vivo. *J. Hazard. Mater.* 298, 310–319.
- Shinohara, N., Danno, N., Ichinose, T., Sasaki, T., Fukui, H., Honda, K., Gamo, M., 2014. Tissue distribution and clearance of intravenously administered titanium dioxide (TiO<sub>2</sub>) nanoparticles. *Nanotoxicology* 8, 132–141.
- Shrivastava, R., Raza, S., Yadav, A., Kushwaha, P., Flora, S.J.S., 2014. Effects of sub-acute exposure to TiO<sub>2</sub>, ZnO and Al2O3 nanoparticles on oxidative stress and histological changes in mouse liver and brain. *Drug Chem. Toxicol.* 37, 336–347.
- Shukla, R.K., Kumar, A., Pandey, A.K., Singh, S.S., Dhawan, A., 2011a. Titanium dioxide nanoparticles induce oxidative stress-mediated apoptosis in human keratinocyte cells. *J. Biomed. Nanotechnol.* 7, 100–101.
- Shukla, R.K., Sharma, V., Pandey, A.K., Singh, S., Sultana, S., Dhawan, A., 2011b. ROS-mediated genotoxicity induced by titanium dioxide nanoparticles in human epidermal cells. *Toxicol. Vitro* 25, 231–241.
- Shukla, R.K., Kumar, A., Gurbani, D., Pandey, A.K., Singh, S., Dhawan, A., 2013. TiO<sub>2</sub> nanoparticles induce oxidative DNA damage and apoptosis in human liver cells. *Nanotoxicology* 7, 48–60.
- Shukla, R.K., Kumar, A., Vallabani, N.V.S., Pandey, A.K., Dhawan, A., 2014. Titanium dioxide nanoparticle-induced oxidative stress triggers DNA damage and hepatic injury in mice. *Nanomedicine* 9, 1423–1434.
- Silva, F., Arezes, P., Swuste, P., 2015. Risk assessment in a research laboratory during sol-gel synthesis of nano-TiO<sub>2</sub>. *Saf. Sci.* 80, 201–212.
- Singh, R., Narwa, H.S., 2011. Medical applications of nanoparticles in biological imaging, cell labeling, antimicrobial agents, and anticancer nanodrugs. *J. Biomed. Nanotechnol.* 7, 489–503.
- Sun, Q.Q., Tan, D.L., Zhou, Q.P., Liu, X.R., Cheng, Z., Liu, G., Zhu, M., Sang, X.Z., Gui, S.X., Cheng, J., Hu, R.P., Tang, M., Hong, F.S., 2012. Oxidative damage of lung and its protective mechanism in mice caused by long-term exposure to titanium dioxide nanoparticles. *J. Biomed. Mater. Res. Part A* 100A, 2554–2562.
- Sund, J., Palomaki, J., Ahonen, N., Savolainen, K., Alenius, H., Puustinen, A., 2014. Phagocytosis of nano-sized titanium dioxide triggers changes in protein acetylation. *J. Proteomics* 108, 469–483.
- Tedja, R., Lim, M., Amal, R., Marquis, C., 2012. Effects of serum adsorption on cellular uptake profile and consequent impact of titanium dioxide nanoparticles on human lung cell lines. *ACS Nano* 6, 4083–4093.
- Thurn, K.T., Arora, H., Paunescu, T., Wu, A.G., Brown, E.M.B., Doty, C., Kremer, J., Woloschak, G., 2011. Endocytosis of titanium dioxide nanoparticles in prostate cancer PC-3M cells. *Nanomed.-Nanotechnol. Biol. Med.* 7, 123–130.
- Traber, M.G., Atkinson, J., 2007. Vitamin E, antioxidant and nothing more. *Free Radic. Biol. Med.* 43, 4–15.
- Trouiller, B., Reliene, R., Westbrook, A., Solaimani, P., Schiestl, R.H., 2009. Titanium dioxide nanoparticles induce DNA damage and genetic instability In vivo in mice. *Cancer Res.* 69, 8784–8789.
- Umbreit, T.H., Francke-Carroll, S., Weaver, J.L., Miller, T.J., Goering, P.L., Sadrieh, N., Stratmeyer, M.E., 2012. Tissue distribution and histopathological effects of titanium dioxide nanoparticles after intravenous or subcutaneous injection in mice. *J. Appl. Toxicol.* 32, 350–357.
- van Ravenzwaay, B., Landsiedel, R., Fabian, E., Burkhardt, S., Strauss, V., Ma-Hock, L., 2009. Comparing fate and effects of three particles of different surface properties Nano-TiO<sub>2</sub>, pigmentary TiO<sub>2</sub> and quartz. *Toxicol. Lett.* 186, 152–159.
- Verbon, E.H., Post, J.A., Boonstra, J., 2012. The influence of reactive oxygen species on cell cycle progression in mammalian cells. *Gene* 511, 1–6.
- Wang, J., Li, Y., Li, W., Chen, C., Li, B., Zhao, Y., 2008a. Biological effect of intranasally instilled titanium dioxide nanoparticles on female mice. *Nano* 3, 279–285.
- Wang, J.X., Chen, C.Y., Liu, Y., Jiao, F., Li, W., Lao, F., Li, Y.F., Li, B., Ge, C.C., Zhou, G.Q., Gao, Y.X., Zhao, Y.L., Chai, Z.F., 2008b. Potential neurological lesion after nasal instillation of TiO<sub>2</sub> nanoparticles in the anatase and rutile crystal phases. *Toxicol. Lett.* 183, 72–80.
- Wang, J.X., Liu, Y., Jiao, F., Lao, F., Li, W., Gu, Y.Q., Li, Y.F., Ge, C.C., Zhou, G.Q., Li, B., Zhao, Y.L., Chai, Z.F., Chen, C.Y., 2008c. Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO<sub>2</sub> nanoparticles. *Toxicology* 254, 82–90.
- Wang, J.X., Fan, Y.B., Gao, Y., Hu, Q.H., Wang, T.C., 2009. TiO<sub>2</sub> nanoparticles translocation and potential toxicological effect in rats after intraarticular injection. *Biomaterials* 30, 4590–4600.
- Wang, J., Li, N., Zheng, L., Wang, S.S., Wang, Y., Zhao, X.Y., Duan, Y.M., Cui, Y.L., Zhou, M., Cai, J.W., Gong, S.J., Wang, H., Hong, F.S., 2011. P38-Nrf-2 signaling pathway of oxidative stress in mice caused by nanoparticulate TiO<sub>2</sub>. *Biol. Trace Elem. Res.* 140, 186–197.
- Wang, J.X., Ma, J.W., Dong, L.M., Hou, Y., Jia, X.L., Niu, X.F., Fan, Y.B., 2013a. Effect of anatase TiO<sub>2</sub> nanoparticles on the growth of RSC-364 rat synovial cell. *J. Nanosci. Nanotechnol.* 13, 3874–3879.
- Wang, Y.L., Wu, Q.X., Stui, K.K., Chen, X.X., Fang, J., Hu, X.F., Wu, M.H., Liu, Y.F., 2013b. A quantitative study of exocytosis of titanium dioxide nanoparticles from neural stem cells. *Nanoscale* 5, 4737–4743.
- Weir, A., Westerhoff, P., Fabricius, L., Hristovski, K., von Goetz, N., 2012. Titanium dioxide nanoparticles in food and personal care products. *Environ. Sci. Technol.* 46, 2242–2250.
- Wilson, C.L., Natarajan, V., Hayward, S.L., Khalimonchuk, O., Kidambi, S., 2015. Mitochondrial dysfunction and loss of glutamate uptake in primary astrocytes exposed to titanium dioxide nanoparticles. *Nanoscale* 7, 18477–18488.
- Wu, J.H., Liu, W., Xue, C.B., Zhou, S.C., Lan, F.L., Bi, L., Xu, H.B., Yang, X.L., Zeng, F.D., 2009. Toxicity and penetration of TiO<sub>2</sub> nanoparticles in hairless mice and porcine skin after subchronic dermal exposure. *Toxicol. Lett.* 191, 1–8.
- Wu, J., Sun, J.A., Xue, Y., 2010. Involvement of JNK and P53 activation in G2/M cell cycle arrest and apoptosis induced by titanium dioxide nanoparticles in neuron cells. *Toxicol. Lett.* 199, 269–276.
- Yamashita, K., Yoshioka, Y., Higashisaka, K., Mimura, K., Morishita, Y., Nozaki, M., Yoshida, T., Ogura, T., Nabeshi, H., Nagano, K., Abe, Y., Kamada, H., Monobe, Y., Imazawa, T., Aoshima, H., Shishido, K., Kawai, Y., Mayumi, T., Tsunoda S.-i. Itoh, N., Yoshikawa, T., Yanagihara, I., Saito, S., Tsutsumi, Y., 2011. Silica and titanium dioxide nanoparticles cause pregnancy complications in mice. *Nat. Nanotechnol.* 6, 321–328.
- Yang, W., Omaye, S.T., 2009. Air pollutants, oxidative stress and human health: mutat. Res. Genet. Toxicol. Environ. Mutagen. 674, 45–54.
- Youdim, M.B.H., Stephenson, G., Ben Shachar, D., 2004. Ironing iron out in Parkinson's disease and other neurodegenerative diseases with iron chelators – a lesson from 6-hydroxydopamine and iron chelators, desferri and VK-28. *Ann. N. Y. Acad. Sci.* 1012, 306–325.
- Zawia, N.H., Lahiri, D.K., Cardozo-Pelaez, F., 2009. Epigenetics, oxidative stress, and Alzheimer disease. *Free Radic. Biol. Med.* 46, 1241–1249.
- Ze, Y., Zheng, L., Zhao, X., Gui, S., Sang, X., Su, J., Guan, N., Zhu, L., Sheng, L., Hu, R., Cheng, J., Cheng, Z., Sun, Q., Wang, L., Hong, F., 2013. Molecular mechanism of titanium dioxide nanoparticles-induced oxidative injury in the brain of mice. *Chemosphere* 92, 1183–1189.
- Ze, Y., Hu, R., Wang, X., Sang, X., Ze, X., Li, B., Su, J., Wang, Y., Guan, N., Zhao, X., Gui, S., Zhu, L., Cheng, Z., Cheng, J., Sheng, L., Sun, Q., Wang, L., Hong, F., 2014. Neurotoxicity and gene-expressed profile in brain-injured mice caused by exposure to titanium dioxide nanoparticles. *J. Biomed. Mater. Res. Part A* 102, 470–478.
- Zhang, R., Niu, Y.J., Li, Y.W., Zhao, C.F., Song, B., Li, Y., Zhou, Y.K., 2010. Acute toxicity study of the interaction between titanium dioxide nanoparticles and lead acetate in mice. *Environ. Toxicol. Pharmacol.* 30, 52–60.
- Zhang, J.C., Cai, X.Q., Zhang, Y., Li, X.M., Li, W.X., Tian, Y.C., Li, A.G., Yu, X.H., Fan, C.H., Huang, Q., 2013. Imaging cellular uptake and intracellular distribution of TiO<sub>2</sub> nanoparticles. *Anal. Methods* 5, 6611–6616.
- Zhang, C.K., Zhai, S.M., Wu, L., Bai, Y.H., Jia, J.B., Zhang, Y., Zhang, B., Yan, B., 2015. Induction of size-dependent breakdown of blood-milk barrier in lactating mice by TiO<sub>2</sub> nanoparticles. *PLoS One* 10, 18.
- Zijno, A., De Angelis, I., De Berardis, B., Andreoli, C., Russo, M.T., Pietraforte, D., Scorza, G., Degan, P., Ponti, J., Rossi, F., Barone, F., 2015. Different mechanisms are involved in oxidative DNA damage and genotoxicity induction by ZnO and TiO<sub>2</sub> nanoparticles in human colon carcinoma cells. *Toxicol. Vitro* 29, 1503–1512.