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Mechanoregulation of titanium dioxide nanoparticles in cancer therapy

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Abstract

Titanium dioxide (TiO_2) nanoparticles (NPs), first developed in the 1990s, have been applied in numerous biomedical fields such as tissue engineering and therapeutic drug development. In recent years, TiO_2-based drug delivery systems have demonstrated the ability to decrease the risk of tumorigenesis and improve cancer therapy. There is increasing research on the origin and effects of pristine and doped TiO_2-based nanotherapeutic drugs. However, the detailed molecular mechanisms by which drug delivery to cancer cells alters sensing of gene mutations, protein degradation, and metabolite changes as well as its associated cumulative effects that determine the microenvironmental mechanosensitive metabolism have not yet been clearly elucidated. This review focuses on the microenvironmental influence of TiO_2-NPs induced various mechanical stimuli on tumor cells. The differential expression of genome, proteome, and metabolome after treatment with TiO_2-NPs is summarized and discussed. In the tumor microenvironment, mechanosensitive DNA mutations, gene delivery, protein degradation, inflammatory responses, and cell viability affected by the mechanical stimuli of TiO_2-NPs are also examined.

Keywords

TiO_2 nanoparticles; Endocytosis; Oxidative stress; Proteogenomics; Metabolomics

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1. Introduction: the physicochemical properties of pristine- and doped-TiO_2-NPs

Titanium dioxide (TiO_2) is abundantly available in nature and possesses specific properties such as biocompatibility, lightness, high corrosion resistance, high thermal stability, low ion release, and non-magnetic properties [1–3]. The current bulk manufacturing of TiO_2 further allows its application in sunscreens, implants, and as a pigment in paint additives. Furthermore, titanium-based nickel alloys are widely used in surgical implants [4]. Recent research has focused on the development of non-toxic and biocompatible tools involving nanoscale titanium, which has a size of less than 100 nm [5]. Particularly, nanoscale TiO_2 particles have unique medicinal properties, including inertia and biocompatibility with body tissues and, thus, have been exploited in many biomedical applications, such as bone plates, dental implants, artificial hips, scaffolds, and coatings, as well as in gene and drug delivery systems. In addition, ultrafine TiO_2-NPs (<100 nm) possess anti-tumor and anti-bacterial properties, showing promise in biomedical fields [6–8].

The geometry and electronic ground state structures of nanoscale $(TiO_2)_n$ groups, where n = 1, 2, 3 and 13, were investigated based on density functional theory (DFT) and time-dependent DFT. Coordination of the titanium atom varies depending on nanoparticle size. In clusters, the average titanium (Ti)–oxygen (O) bonding distance is smaller than that in bulk materials, which results in more solid-like structures. In case of bulk crystals, differences in composition, size/diameter, shape of clusters, and amount of TiO_2 units can cause sensitive variations in the electronic structure and energy gap [9, 10].

TiO_2 acts as a photocatalyst and is synthetically available in three different forms: rutile (tetragonal), anatase (tetragonal), and brookite (orthorhombic) (Figure 1) [9, 11, 16]. Rutile TiO_2 is more stable than the anatase and brookite forms, and anatase TiO_2 shows a larger bandgap (3.2 eV/nm vs. 2.0 eV/nm of rutile TiO_2) and greater particle surface area than rutile TiO_2 [11, 12]. The adsorption of TiO_2 through **π-π** stacking is essential for titanium loading into other biomaterials such as titanium-implanted alloys. On the surface of TiO_2 nanotubes, bound-intermediate transfer reactions occur, similar to H-H, C-H, C-O, O-H, C=C, and C=O bonds, including van der Waals forces [13, 14]. When anatase TiO_2-NPs are exposed to ultraviolet (UV) radiation, an electron can get excited from the valence band to the conduction band via the band gap in the photocatalyst [15, 16].

Transmission electron microscopy (TEM) images showed that dispersion of agglomerated brookite TiO_2-NPs in either polar (water) or non-polar (cyclohexane) solvents led to changes in the morphology of the NPs due to amphiphilic properties [17, 18]. The aggregation or agglomeration of sphere-shaped NPs also occurred, as indicated by the large size of the particles in the presence of ionic liquids (ILs) [19, 20]. In addition to TEM, field emission transmission electron microscopy (FETEM) has been used for the morphological analysis of pristine and Ag-doped TiO_2-NPs, which indicated that Ag-doping reduces the size of the host-TiO_2-NPs [21, 22]. X-ray powder diffraction (XRD), another analytical technique, has been reported to be essential to determine the crystal structure in ionic liquids (ILs) and assess the crystal grain size using the Scherrer equation [23]. However, due to the detection limit of XRD, sizes smaller than 3–4 nm cannot be estimated. In addition, Raman

spectroscopy has been used to examine structural changes in the anatase and rutile forms of TiO_2 [24, 25].

The photo-irradiation of anatase TiO_2-NPs can generate electron-hole pairs that improve photocatalytic performance. Based on the photocatalytic process, these electrons and holes can react with oxygen molecules (O_2) dissolved in aqueous solution or water molecules (H_2O) absorbed on the surfaces of TiO_2 particles, respectively [26–28]. Subsequently, this process can produce reactive oxygen species (ROS), such as short-lived free hydroxyl radicals ($^{\bullet}OH$), in different aqueous media. Recently, ROS-mediated anticancer treatment, such as photodynamic therapy (PDT), has demonstrated improved site-specific activities [29–32].

Inhaled ultrafine nanoparticles of TiO_2 are generally regarded to be biologically inactive and physiologically inert. However, translocation of these ultrafine TiO_2-NPs into human lung cells was observed after inhalation. Translocation of the ultrafine TiO_2-NPs from the lung surface into the tissue has been observed to a greater extent than larger sized TiO_2-NPs. This phenomenon has also been observed in a patient, as previously reported [33, 34]. Recently, high concentrations of pigment-grade (<2.5 μm) and ultrafine (<100 nm) TiO_2-NPs have been reclassified as “possibly carcinogenic to human beings” by the International Agency for Research on Cancer (IARC, 2006) [35–37]. Indeed, this opinion has been confirmed by several studies, which showed that high concentrations of ultrafine TiO_2 could induce rat lung tumors *in vivo* [38–41].

Compared to pristine NPs, doped-TiO_2-NPs elicited substantially greater inflammatory responses in mice [42] and zebrafish [43]. Therefore, the development of effective anti-inflammatory strategies remains an important area of research. In contrastingly, understanding the microenvironment and physiology of tumor cells upon exposure to TiO_2-NPs and doped-TiO_2 could provide support for immunotherapy and cell viability analyses. The toxicity (i.e., cytotoxicity and genotoxicity) and impact of these NPs in aquatic ecosystems have been previously discussed in the literature [44, 45]. In this review, we delineate the existing knowledge of pristine and doped-TiO_2-NPs with regard to their chemotoxicity (including ecotoxicity, cytotoxicity, and genotoxicity) and mechanobiological influences (genome-, proteome-, and metabolome-wide) and highlight the circadian rhythmicity in tumor microenvironments.

2. Toxicity of TiO_2-NPs

The cytotoxic and genotoxic effects exerted by different types of TiO_2-NPs on aquatic vertebrates and invertebrates have been listed in table 1. With similar genetic structure to humans, zebrafish (*Danio rerio*), goldfish (*Carassius auratus*), and water fleas (*Cladocera*) are popular models for drug-screening, gene therapy, or other biomedical applications in NP-based drug development. Furthermore, both zebrafish and goldfish are easy to breed with fairly low maintenance costs [46–49].

The ecological toxicity of NPs in marine animals is typically measured in terms of acute (maximum 2 weeks/14 days), sub-acute (maximum 4 weeks/28 days), sub-chronic (13

weeks/90 days), or chronic (more than 4 months) exposure [50, 51]. Ramsden et al. showed that, when TiO_2-NPs were dispersed in water, 30% of NPs were deposited at the bottom of the aquarium tank. However, the remaining particles were sufficiently concentrated to cause toxicity to the aquatic organisms. Moreover, generation of reactive oxygen species (ROS) and depletion of intracellular glutathione (GSH) led to damaged cellular components and affected metabolism [52, 53]. Zhu et al. claimed that TiO_2-NPs showed low ecological toxicity in water fleas up to 48 h after exposure [54]. However, photo-induced ecotoxicity of TiO_2-NPs was observed at 72 h and 21 days of exposure in aquatic organisms such as *Daphnia magna* and medaka. Anatase TiO_2-NPs may cause growth inhibition that lowers reproduction and increases mortality [55, 56]. The acute cytotoxic effects of six different nanomaterials (TiO_2; zinc oxide, ZnO; aluminum oxide, Al_2O_3; fullerene, C_{60}; single-walled carbon nanotubes, SWCNTs; and multi-walled CNTs, MWCNTs) were carefully analyzed in water fleas (48 h post treatment) with regard to mortality and toxicological endpoints (lethal concentration, LC_{50}) [57]. The study demonstrated that these manufactured nanomaterials were more toxic than their bulk counterparts, suggesting their potential hazardous environmental effects. P25 (Degussa) TiO_2, or peroxide TiO_2, increases immobilization and toxicity upon UV light exposure, which could be attributed to the generation of ROS [58, 59].

In zebrafish, sub-chronic exposure (13 weeks) to anatase TiO_2-NPs at low concentrations (e.g., 0.1 mg/L) severely damaged the reproductive system and downregulated gene expression (mRNA level) [60, 61]. Sub-chronic exposure to TiO_2-NPs induced mechanical stress in cells, resulting in the downregulation of energy-dependent enzymes, such as Na^+/K^+ ATPase. Accordingly, zinc and copper (Zn–Cu) concentration gradients across the cell membrane are reduced by exposure to both pristine and doped TiO_2-NPs [51]. Ultrafine TiO_2-NPs (~20 nm in size), copper (Cu)-TiO_2-NPs, and silver (Ag)-NPs were found to affect many immune response genes. For example, 37 upregulated (*jdp2, gata6, fosb, fos, mgat5, nrxn3b*, etc.) and 33 downregulated (*wt1a, cry5, gata4, glis3, ahsg*, etc.) genes related to immune modulation were identified in zebrafish embryos upon TiO_2 exposure. It has been reported that TiO_2-NPs significantly affected liver tissue metabolism in a dose-dependent manner (< 0.1 mg/mL) in freshwater goldfish and zebrafish [61–63]. Similarly, Nowack-Bucheli and Chen et al. reviewed the effects of TiO_2-NPs with regard to environmental behavioral alteration, risk assessment, and emerging contaminants [64, 65].

Nanotoxicity of pristine and functionalized TiO_2-NPs in freshwater organisms, such as algae, zebrafish, and other invertebrates, has been collectively summarized [66]. Particularly, genetic variations and functional relationships among genes associated with calcium ions, mitosis, apoptosis, oxidative stress, inflammation, and ROS have been identified using different concentrations of TiO_2-NPs [67]. Both pristine and functionalized TiO_2-NPs caused DNA strand breaks (including oxidative damage to DNA) and damaged lysosomal membrane integrity and DNA-protein complexes [68–71]. Exposure of rainbow trout (*O. mykiss*) to TiO_2-NPs induced oxidative stress (OS), a primary outcome of toxicity; moreover, increased genotoxic effects were observed upon exposure to a mixture of rutile and anatase TiO_2-NPs [51]. Anatase TiO_2-NPs induce genotoxic effects by causing oxidative DNA damage in aquatic species. Recent studies have examined the potential ecological impact on gene expression and metabolic oscillations of aquatic species upon exposure to

TiO_2-NPs [68, 69]. The ecotoxicity, cytotoxicity, and genotoxicity of TiO_2-NPs are all key considerations in terms of potential clinical translations.

3. Antitumor effects of TiO_2-NPs and their underlying mechanisms

NPs may cause numerous non-specific effects in cellular microenvironments and subcellular organelles. The investigated antitumor effects of pristine and surface modified TiO_2-NPs with various diameters have been summarized in table 2. NP size, aggregation tendency, and agglomeration are key factors that determine cell viability and genetic alteration in tumor cells. Increased NP diffusion in tumor microenvironments transforms the cell structure [21, 72]. Thus far, the effects of metal oxide NPs on tissue physiology and cellular microenvironment have not been well studied. An in-depth understanding of the anticancer activity via mechanical stimuli is essential for developing effective therapies [73–76].

Several metabolic pathways perform important functions in the cellular uptake of TiO_2-NPs [80]. In contrast, TiO_2-NPs also alter the cellular mechanistic stress-dependent signaling pathways mediated by MAPK activation (ERK, JNK, and p38) and subsequent transcription factor activation (NF-kB, Nrf2, etc.). TiO_2-NPs enter the cell via pinocytosis, phagocytosis, or micropinocytosis and, then, get accumulated in the cells at specific locations, such as the vesicles, cytoplasm, or mitochondria [77– 81]. Endocytosis occurs at the plasma membrane to capture extracellular nanoparticles through natural vesicular secretions. Phagocytosis is the preferred mode of uptake of particles larger than 500 nm. Nevertheless, larger NPs (micrometer-sized) can also be taken up by phagocytosis with high efficiency. This mechanism allows cells to feed and defend themselves as well as regulate homeostasis. Pinocytosis occurs when cells encounter smaller NPs (2–6 nm) and is often initiated and mediated by cell surface receptors binding to target ligands [82–84]. The pinocytic vesicles, formed by either clathrin-dependent or independent mechanisms, subsequently fuse with endosomes. The internalization mechanism can vary based on different particle sizes ranging from a few to several hundred nanometers. This penetration of TiO_2-NPs reorganizes the cell architecture [85]. It has been reported that the increased levels of hydrogen peroxide (H_2O_2), hydroxyl radical ($^{\bullet}OH$), superoxide ($O_2^{\bullet-}$), hydroperoxyl ($HO_2^{\bullet}$) and cell sensitization upon TiO_2-NPs exposure led to reduced cell proliferation rate, organ-specific carcinogenesis, and mortality [86–88].

Studies on TiO_2 nanoformulations also demonstrated a potential mechanism that affected cell proliferation by blocking the cell cycle. Cell division involves numerous DNA checkpoints in each phase (e.g., Gap1, G1 phase; DNA proliferation, S phase; Gap 2/ mitosis, G2 phase) [77]. TiO_2 nanofilaments exhibited enhanced cytotoxic action and a strong dose-dependent effect on cell proliferation and cell death [89]. Additionally, TiO_2-NPs showed cell cycle disruption similar to that caused by nanofibers; it has been reported that 12.8% of cells were blocked at the sub-G1 phase upon exposure to 5% Ag-doped TiO_2-NPs [21]. Hence, DNA replication might be inhibited due to asymmetric cell division. The G2/M phase was also weakened upon treatment of cells with Ag-doped TiO_2-NPs [61, 90, 91].

Redox chemical reactions (i.e., oxidation and reduction) occur when TiO_2-NPs are exposed to UV rays. During the redox process, electrons and holes are produced, which can strongly react with surrounding O_2 and H_2O molecules and, finally, generate numerous ROS (e.g., $^{\bullet}OH$, $O_2^{\bullet-}$, peroxide (O_2^{2-}), singlet oxygen ($^1O_2$), and H_2O_2 [73, 74]. Among these, $O_2^{\bullet-}$ and H_2O_2 are sensitive oxidation carriers. The diffusion rates of these two radicals can reach substantially higher levels than that of other ROS. The $O_2^{\bullet-}$ molecules can quickly penetrate and influence subcellular organelles (nuclei, mitochondria, and others), ultimately governing cellular function [75, 76]. ROS also has different redox properties and is involved in several biochemical reactions with the lipid bilayer and DNA molecules that could eventually kill cancer cells. Additionally, a small amount of ROS plays an important role in the disruption of cellular homeostasis. Thus, as a cellular signaling messenger, ROS modify protein topography and cause proteome deterioration [77–79]. Intracellular ROS levels can be monitored using 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) and dihydroethidium (DHE) to better understand the cytotoxic effects. Fluorescence microscopy images at 24 and 48 h post-TiO_2-NP treatment implied that HCT-116 (colorectal carcinoma cells) cell death occurred by either apoptosis or necrosis [21, 92]. The induction of apoptosis was observed post 5, 10, and 15 h of TiO_2-NP treatment; further, quantification of apoptotic cells suggested that cell proliferation was inhibited. In addition, ATP levels suggested that some degree of necrosis occurred but not apoptosis. It has been reported that treatment with TiO_2-NPs led to the depletion of ATP levels, thereby inhibiting apoptosis and causing necrotic cell death [27, 28]. A previous study has reported that, in cellular microenvironments, ROS triggers nucleotide damage due to its high reactivity and can regulate cytoplasmic calcium (Ca^{2+}) levels [45, 92], potentially causing epithelial damage and respiratory toxicity over an extended period [93]. When TiO_2 enters the cell, the conditions become acidic (pH 4.5); thus, it affects several biological events such as proton leaks, membrane trafficking, and proton pump (ATPases) activity [94].

In non-tumor cells, metabolic stress leads to the disruption of proinflammatory cytokine network- (e.g., glutathione metabolism and nicotinate-nicotinamide metabolism) and age-related diseases. This reveals the potential mechanisms of the NP-mediated toxicity [95]. Tumor necrosis factor-beta (TNF-β) levels were increased in fish embryos exposed to both 12–14 nm and 150–200 nm TiO_2-NPs. TNF-β is a cytokine that acts as a potent mediator of proinflammatory responses and tumoricidal activities [96–101]. Neutrophil influx and protein levels in bronchoalveolar lavage fluid (BALF) and ROS activity were increased in TiO_2-NP-treated tumor cells, demonstrating the ability of TiO_2-NPs to regulate the expression of inflammatory genes [77]. Antioxidant enzymes, such as superoxide dismutases (SOD1 and SOD2), can act as the primary defense against NP-induced stress. After TiO_2 exposure, SOD converts superoxide ($O_2^{\bullet-}$) radicals to hydrogen peroxide (H_2O_2) [102, 103]. Increased ROS levels may impair metabolic pathways and molecular processes and reduce cellular lifespan. Hypoxia, driven by pristine and functionalized TiO_2-NPs, could have tremendous effects on aging, metabolism (purine and pyrimidine metabolism), and the immune system [104–109].

Mitochondrial apoptosis, independent of the caspase 8/t-Bid pathway, was assessed in human bronchoalveolar carcinoma-derived cells (A549) and human bronchial epithelial cells (BEAS-2B) treated with metal oxide NPs, such as TiO_2-NPs, Fe_2O_3, Mn_2O_3, Cr_2O_3, NiO,

CuO, and ZnO [102, 110, 111]. These NPs were reported to be physicochemical inducers of apoptosis via caspase-3, caspase-8, and caspase-9 signaling in mitochondrial pathways (i.e., intrinsic and extrinsic pathways). The activation of these apoptotic signaling pathways depends on the internalization of TiO_2-NPs and their reactivity. Changes in cell potency and biphasic system formulations were observed in human liver cancer (HepG2) cells treated with undoped or doped TiO_2-NPs, which influenced mitochondrial permeability and lysosomal activity [21, 112]. To better understand NP-mediated toxicity mechanisms, the following methods have been widely applied to cells to examine metabolic stress, regulation of inflammatory gene expression, and carcinogenic effects.

Cell viability and LDH activity

Among the different cancer diagnostic methods, metabolic cytotoxicity (loss of viable cells) has been assessed using various combinations of TiO_2 particles. As a result, black TiO_2, functionalized-TiO_2-NPs, doxorubicin (DOX)-TiO_2 nanocomposites (NC), and folic acid conjugated (NC-FA) with photothermal therapy (PTT) decreased the viability of MCF-7 cells. In contrast, pristine TiO_2 did not decrease the viability of MCF-7 cells [21, 105, 155]. Doped-TiO_2-NPs were found to induce cytotoxicity and cell cycle arrest in various tumor cells. Metabolic cytotoxicity responses induced by TiO_2-NPs were used as diagnostic tools in clinical chemotherapy. Human lung carcinoma (A549) and breast cancer cells (MCF-7) treated with TiO_2- and Ag-TiO_2-NPs exhibited reduced cell viability, thereby indicating the chemotherapeutic effects of these NPs [95, 104, 133]. *In vitro* cell viability of HeLa cells was reduced upon exposure to 800 nm NIR laser irradiation with various doses of black-TiO_2-x. Moreover, brain cancer cells (U87MG and PC12) were incubated with black-TiO_2-x and the percentage of cell viability was evaluated. No cytotoxic effects were observed, and the percentage of cell viability was above 85% in these cells [156]. In pancreatic cancer stem-like cells (PANC-1 cells), *in vitro* cytotoxicity of black TiO_2-based nanoprobes expressing CD133 monoclonal antibodies (black-TiO_2-Gd and black-TiO_2-Gd-CD133), was examined for PTT of pancreatic cancer. The applied nanoprobes exhibited active targeting ability in PANC-1 cells [157]. Based on the low cytotoxicity observed in pancreatic cancer PTT, cancer therapeutic effects of hydrogenated black-TiO_2 exposure in MCF-7 and 4T1 cells was demonstrated [158].

In the tumor microenvironment, release of lactate dehydrogenase (LDH) can directly damage the cell membrane. The catalytic conversion of NAD^+ to NADH is directly proportional to LDH activity; thus, it has now been widely used to determine LDH expression. No changes were observed in pure TiO_2-NP-treated cells [21, 107]. In normal and cancer cells treated with Ag-TiO_2-NPs (5%), the LDH levels were found to be increased by approximately 50% compared to untreated cells. The LDH level was also shown to be increased in cells treated with Ag-TiO_2-NPs compared to those treated with pure TiO_2 [109, 113]. In fact, low exposure to pristine TiO_2-NPs (0.5–200 μg/mL) did not cause increased LDH activity in both assays. Experimental results showed decreased cell viability of Ag-coated TiO_2-NPs as the dosage was gradually increased. It has been reported that Ag-coated TiO_2-NPs induced high leakage of LDH in HepG2 cells, whereas pure TiO_2-NPs did not show such induction. The morphology of HepG2 cells was also considerably altered by Zn-doped TiO_2-NPs, with lower cell density compared to that of the control cells [105].

DNA bridging-breakage by TiO_2-NPs

Alkaline single-cell gel electrophoresis (comet assay), a sensitive method for the detection of DNA damage in cells, has been used to test genotoxicity of novel chemicals and nanomaterials. Cell cycle checkpoints, such as the G2/M phase, were not activated in tumor cells treated with pristine TiO_2 or doped TiO_2-NPs [114, 115], resulting in the inhibition of cell division. The genome rearrangements induced by TiO_2-NPs were observed in different cellular microenvironments, leading to considerable DNA damage. A recent study reported that TiO_2-NP exposure caused DNA damage, such as single-stranded breaks, double-stranded breaks, and DNA-DNA crosslinking. Therefore, DNA fragmentation might occur due to TiO_2 exposure, which could activate DNA-damaging anticancer agents [115].

Chromosomal breakage induced by TiO_2-NPs

The cytokinesis-blocked micronucleus (CBMN) assay is a technique that measures the mechanistic basis of chromosomal instability, chromosomal loss, and non-disjunction that occurs due to TiO_2-NP exposure. This cytogenetics approach specifically scores micronuclei (MNi) in mononucleated cells. This method has been effectively applied to evaluate nuclear dysfunction, aging, micronutrient deficiency or excess, exposure to genotoxins, and genetic defects that significantly affect maintenance of genome stability [116, 126]. Rutile TiO_2-NPs were shown to trigger increased chromosomal damage, through sister-chromatid rupture and DNA-bridging, more effectively than other types of NPs. More importantly, *in vivo* genetic instability and delayed cell proliferation are the underlying mechanisms of carcinogenesis caused by TiO_2-NP exposure. These chromosomal rearrangements and genetic instability highlight the possible survival of genomically abnormal cells [89, 117].

4. Precision oncology of TiO_2-NPs-based genomics and proteomics

In tumor spheroids, TiO_2-NPs can be efficiently absorbed by proteins in the cytoplasm, which may cause proteogenomic disruption (Figure 2). This could happen nonspecifically and lead to numerous perturbances inside the tumor microenvironment. Thus far, only a few of the altered proteins have been identified [25]. Western blot/immunoblotting analysis revealed that protein levels of SOD1, SOD2, heme oxygenase-1 (HO-1), and β-actin were significantly impaired in human breast cancer MCF-7 cells treated with pristine or Zn-TiO_2-NPs [104, 118]. The level of HO-1 protein was increased after TiO_2-NP treatment, indicating its poor degradation compared to the control. The expression and mechanisms, such as chromothripsis or kataegis, of these heterogeneous proteins were comprehensively assessed by immunoblotting analysis. The results suggested that Zn-TiO_2-NPs diminished SOD1 and SOD2 expression, which was correlated to the sensitivity and interlinkage of TiO_2-induced protein-protein degradation with the interactive association of other proteins [104, 118]. In contrast, pristine TiO_2 did not exhibit any effect on the protein levels of SOD1 or SOD2, which resulted in lower levels of intracellular toxicity in MCF-7 cells compared to cells treated with Zn-doped TiO_2-NPs. Besides, Zn-TiO_2-NPs induced higher expression of N-acetyl-cysteine (NAC), which acts as an ROS scavenger and significantly inhibited ROS generation [116]. In addition to the mechanistic stimuli induced by TiO_2-NPs in the tumor microenvironment, the reduction of antioxidant metabolites (i.e., glutathione (GSH), taurine, betaine) and inhibition of antioxidant enzymes (GSH peroxidase (GPx), catalase, SOD1,

SOD2, and Nrf2) have also been linked to different defense mechanisms in cancer metabolism. The caspase family of genes, e.g., caspase-3, 8, and 9, which play critical roles in regulating cell-death, were activated during the process of necrosis. A previous study demonstrated that activation of caspase genes is imperative for genetic damage and programmed cell death [108]. Ag-functionalized TiO_2-NPs could entirely inhibit caspases and metacaspases, which highlights the death-centric role of caspases. Especially, a high dose of Ag-doped TiO_2-NPs affects the enzyme activity of caspase-3, which is a frequently activated death protease, by catalyzing specific proteins [104].

Among the molecular transporters in tumor spheroids, antioxidant enzymes of the peroxiredoxin family (PRX, e.g., Prx1, 3, 4, and 5) can be altered by metal oxide NPs [119]. In HeLa cells, Prx1 expression was not detected in the presence of high concentrations of TiO_2-NPs. However, at lower concentrations of TiO_2-NPs, Prx1 expression was more evident, compared to the expression of the multi-functional protein actin (used as a housekeeping control) [119]. PCR analysis data of PRX family genes was validated by western blot analysis after specific TiO_2-NP exposure. Among these, an antioxidant enzyme was identified in mammalian cells, encoded by four genes (i.e., *PRDX1*, *PRDX3*, *PRDX4*, and *PRDX5*), all of which belonged to the peroxiredoxin (PRDX) family. Summarized results of the PCR assays revealed the genomic toxicity and metabolic pathways of the PRDX family genes following TiO_2-NP exposure [120].

Immunoblotting analysis of cells treated with pristine TiO_2-NPs (20, 40, and 80 μg/mL) revealed that the levels of heat shock proteins (HSPs; Hsp60, Hsp70) and apoptotic proteins (p53, BAX, Bcl-2, Apax-1, caspases-3, caspase-9) were increased after NP exposure. A recent study using 3D-cultured tumor spheroids demonstrated that caspase-3 and caspase-9 activation after TiO_2 treatment led to a cascade of events that triggered cell death or inhibited proliferation of tumors [121]. Apart from caspase activity, HSP proteins are also potential targets for cancer therapy. It should be noted that HSP60- and HSP70-dependent molecular pathways failed to stabilize chaperone complexes [121–123].

Numerous studies have been carried out to identify chemical and biological stimuli responsible for altered Bcl-2 expression in pro- and anti-apoptotic protein metabolism. According to a recent study, elevated expression levels of BAX (pro-apoptotic) and reduced expression levels of Bcl-2 proteins (anti-apoptotic) were observed following treatment of cells with TiO_2. Due to changes in the BAX/Bcl-2 ratio, the levels of the tumor suppressor protein p53 might be increased, thereby indicating that it plays an important role in the response to chemotherapy and stimulation of specific defense enzyme activities and their related genes (e.g., CAT, SOD, GSTs from adult zebrafish organs) [124]. Apoptosome formation can be activated by apoptotic protease-activating factor (Apaf-1). The endocytosis-mediated genes, such as *elmod2, sh3bp4, jmjd6, sh3bp4, and zgc*: *101777*, have shown elevated expression in zebrafish embryos exposed to TiO_2 particles. Meanwhile, TiO_2 nanomaterials adversely affected cytoskeletal genes and their corresponding proteins, indicating their toxic effects on zebrafish embryogenesis [125].

VE-cadherin or the cadherin 5-actin ternary complex is essential for structural maintenance of cell shape and junction stability [122]. A study proposed that the physical interaction of

TiO_2-NPs with VE-cadherin could induce mechanistic stress that leads to actin rearrangement and changes in the shape of the plasma membrane or homophilic impairment. The small sized TiO_2 particles (~50 nm) disrupted the endothelial cell leakiness (ECL) and major adherens junctions. Actin dysregulation might induce variations in the cellular phenotype, causing leakage between neighboring cells via cell-cell interactions [127]. In contrast, TiO_2 nanoparticles with a larger size (~650 nm) did not affect ECL when bound to VE-cadherin. The localization and degradation of VE-cadherin is associated with catenin proteins such as p120 and β-catenin. The loss of VE-cadherin rigidity or even denaturation of VE-cadherin might inhibit homophilic communication. Studies have demonstrated that strong interactions between cells and TiO_2-NPs induced ECL, leading to the separation of endothelial cells from each other as observed by visualization of nuclei and VE-cadherin at adherens junctions [128–130].

Increased MCP-1 (CCL2) protein level and enhanced interleukin 8 (IL-8) production have been observed in THP-1 and A549 cells at different time points after treatment with TiO_2-NPs. The inflammatory molecules, IL-1ß, IL-8, IFN-γ, and tumor necrosis factor (TNF)-α, were sensitive to porous TiO_2-NPs [86, 95]. The expression of these inflammatory factors affects transcription oscillation and cytokine activity and is attributed to the pathogenesis of inflammatory bowel disease (IBD); thus, they could be therapeutic targets for IBD. TiO_2 could block neoplastic lesions owing to inadequate inflammatory responses [96]. Long-term exposure to pristine and functionally modified TiO_2-NPs may increase Ca^{2+} influx into the extracellular environment through membrane L-type Ca^{2+} channels and elevated expression of the PKC/p-38 MAPK cascade, ultimately activating NF-κB [97, 98]. TiO_2-NPs may disintegrate cancer cell integrins, leading to the initiation of apoptosis and affecting metabolic pathways. Intracellular Ca^{2+} signaling was modulated in TiO_2-treated cells, which, in turn, altered cellular electrophysiology and immune responses. Moreover, as evidenced from prior publications, pristine- and doped TiO_2-NP-treated cells could change from a normoxic state to a hypoxic state [131].

The effects of TiO_2-NP exposure on several genes and proteins *in vitro* are summarized in table 3. Important clinical biomarkers such as SOD2, PRDX3, Hsp60, BAX, Bcl-2, IL-8, caspase-3, and VE-cadherin suggest that proteolysis, cytokine activity, and apoptosis should be considered in different microenvironmental areas (i.e., cytosol, mitochondria, nucleus, plasma membrane, etc.) in various cancer cells. TiO_2-NPs have shown great promise in controlling tumor proliferation and gene mutations and blocking inflammatory factors [132–134]. Apoptosis-like and necrosis-like programmed cell death was observed in U87 (human astrocytoma) and HFF1 cells (human fibroblasts) treated with TiO_2-NPs [135, 136]. Moreover, exposure to nanoscale ZnO- and TiO_2-NPs has been shown to cause aberrations in genome sequences [99, 104, 109, 153]. Collectively, the effects induced by TiO_2-NPs in the plasma membrane, mitochondria, nucleus, and cytosol of tumor cells demonstrate their potential in anticancer therapies.

5. Prognostic and predictive metabolite rhythmicity by TiO_2-NPs

The metabolome (quantification of metabolites) is a collection of metabolites produced by the cells, which forms a network of biochemical reactions that indicates cellular activity and

physiological status. TiO_2-NP-based target profiling and metabolite variations have been investigated in the context of metabolomics, which shows great promise in investigating changes in metabolic pathways. At the systemic level, metabolomics provides insights regarding whole cell response to nanoparticles [137, 138]. Consequently, after low-dose or high-dose exposure to TiO_2-NPs, intermediate changes in metabolites were detected in several pathways involved in energy release (ATP; TCA cycle), amino acid (glutathione) metabolism, and enzyme (NAD; acetyl-CoA) and membrane structures (MMP) (Figure 3). In particular, the outputs of glucose, pyruvate, nicotinamide adenine dinucleotide (NAD^+, a vital co-factor in the glycolytic pathway), NADH, NAD phosphate ($NADP^+$), glutathione (GSH), methionine, acetyl-CoA, and S-adenosylhomocysteine (SAH) were significantly altered [3, 139], whereas the cellular redox ratio of reduced-to-oxidized NAD and FAD regulated protein oscillation functions. Although TiO_2-NPs have been studied more specifically in the tumor microenvironment, they have been linked to the NAD salvage pathway, redox homeostasis, neurodegeneration, and aging [106].

Treatment of HaCaT cells with pure TiO_2-NPs at a dose of 100 μg/mL caused a significant reduction in some essential metabolic enzymes, such as NAD^+, NADH, and $NADP^+$. The disrupted NAD^+/NADH equivalence might hinder the GSH (GSH/GSSG) and Krebs cycles. The inhibited GSH pathway might be unable to defend against mechanobiological stress and altered osmotic pressure [106, 119, 140]. Additionally, the Krebs cycle and glycolytic pathways might be impaired upon TiO_2-NP exposure. Reduction of acetyl-CoA, carnitine, and acetyl-carnitine levels can modify the oxidation of fatty acids and the Krebs cycle. Due to acetyl-CoA imbalance caused by TiO_2, acetylation impairs the catalytic activities of many other enzymes (i.e., cell-cycle regulating kinases CDK1, CDK2, and CDK5). The physicochemical properties of GSH and NAD^+ are largely altered upon exposure to TiO_2-NPs. These metabolite-phenotypic states could generate stressful microenvironments upon exposure to high doses of TiO_2-NPs [98, 141, 142].

The metabolites of S-adenosylmethionine (SAM) and SAH regulate the conversion of methionine to homocysteine. The biosynthesis of SAM occurs by methionine adenosyltransferase, which utilizes ATP [98, 100]. TiO_2-NP treatment might hinder the synthesis of SAM as well as its utilization and regeneration. SAM serves as a methyl donor to many chemical reactions that yield SAH. However, these biochemical reactions could be inhibited at various steps of synthetic pathways. Due to this blockage of SAH and SAM in TiO_2-NP-treated HaCaT cells, biological reactions involving conversion of methionine to GSH might be inhibited. The downregulated homocysteine may bind to adenosine to form SAH in the presence of SAH hydrolase. Therefore, SAH-mediated hydrolytic process is hampered in the presence of higher dose of TiO_2-NPs [143, 144].

The sulfur-containing amino acid methionine plays an important role in cellular transcriptional regulation and DNA methylation. It acts as an initiator of translation of polypeptides. Methionine is required for transcription and translation and can promote normal and neoplastic cell division [145]. Downregulated methylation and GSH synthesis have been associated with increased oxidative stress. Therefore, TiO_2-NP treatment hinders methionine-based epigenetic modification (e.g., methylation, acetylation, phosphorylation),

which, in turn, causes a shortage of methionine in tissues, thereby inducing carcinogenesis [146].

Reduced levels of trimethylamine N-oxide (TMAO) were detected in aquatic animals exposed to TiO_2-NPs. The TMAO level differs according to cell type in marine animals [139]. TMAO acts as a primary stabilizer for proteins and nucleic acids, protecting the cellular environment from hydrostatic pressures. *In vitro* analysis showed fluctuations of TMAO levels in pristine TiO_2-NP-treated mouse fibroblast (L929) cells [147, 148].

Some more relevant amino acid metabolic pathways affected by TiO_2-NPs were identified based on higher p-values and impact values interrelated with several other metabolic pathways. Pathway enrichment and topology analyses of all corresponding metabolic pathways (including alanine, aspartate, and glutamate metabolism; cysteine and methionine metabolism; beta-alanine metabolism; glycine, serine, and threonine metabolism; lysine degradation; and glutamine and glutamate metabolism) has been reported [139]. High doses of TiO_2-NPs inhibited nucleotide metabolism (i.e., purine and pyrimidine metabolism) either directly or indirectly. More importantly, these NPs could block DNA proliferation, RNA transcription, and protein synthesis in L929 mouse fibroblast cells, which are widely used for toxicity analysis. The pristine TiO_2-NPs affected amino acid metabolism (such as the Krebs cycle intermediates, traditionally associated with bioenergetics or biosynthesis) and nucleotide metabolism [149, 150]. Currently, metabolic reprograming and metabolomic profiling are key areas of research to understand the underlying mechanisms of carcinogenesis.

6. Conclusions and outlook

Benefiting from recent advances in multi-omics technologies (genomics, proteomics, and metabolomics), TiO_2-NP-treatment induced mechanical stimuli are now being better understood in tumor cell biology. There is increasing research regarding the commercial use of pristine and functionalized TiO_2-NPs for various drug-gene therapies. With their extraordinary rigidity and biocompatibility, TiO_2-NPs have secured a special place in biomedical implantation. In particular, TiO_2-NP-mediated multi-omics technologies have universally matured to target structural, molecular, and phenotypic variations. Currently, several types of functionalized TiO_2-NPs are available as promising platforms for tumor diagnosis and treatment.

In this review, we summarize the synergistic effects of TiO_2-NPs on biological reactions in the context of various influences in the tumor microenvironment. Omics functional analysis showed that gene mutations, protein degradation, and metabolite changes induced by TiO_2-NPs varied in different species, depending on particle sizes, routes of exposure, and dispersion solvents. More importantly, most of the DNA/RNA-based diagnostic studies are based on NP sizes, concentrations, and exposure periods. The putative molecular mechanisms of oxidative stress proteins (SOD1, SOD2, HO-1, PRDX family), inflammatory proteins (β-actin, IL-1β, IL-8), junction protein (VE-cadherin), Krebs cycle intermediates (glucose, pyruvate, NAD^+,), enzymes, and amino acid metabolites (GSH, methionine, etc.,) have been outlined. From these analyses, pristine TiO_2 shows fewer antitumor effects than

doped-TiO_2, both *in vitro* and *in vivo*, either by direct action on the tumor cells or indirectly via ROS. In particular, the presence of pristine and surface-modified-TiO_2 particles enhanced $^{\bullet}OH$, $O_2^{\bullet-}$, and $^1O_2$ radical production, even in the absence of light. These radicals can alter metabolic pathways in the powerhouse of the cells (inside and outside the mitochondria) and cause electron-transfer reactions that trigger programmed cell death (apoptosis). There are very few reports regarding *in vivo* toxicology profiling of pristine and surface doped-TiO_2-NPs in zebrafish, which may not be sufficient for clinical translation. At present, the robust and sensitive assays (i.e., MTT-assay, LDH-assay, real-time PCR, and western blotting) that are being used in molecular imaging might be essential for assessing their therapeutic efficacy and identifying specific clinical biomarkers of diseases [151, 152,].

Most recent publications indicate that pure and Au-TiO_2-NPs, Zn-TiO_2-NPs, Ag-TiO_2-NPs, as chemotherapeutic drugs or nanomedicines, are genotoxic and could be applied for treating various cancers. In addition, the radioactive probes bound to black-TiO_2 can be used to treat deep cancer tissue more efficiently [159, 160]. Assessing complex microenvironmental modulation by TiO_2-NP treatment both *in vivo* and *in vitro* is necessary to discover biomarkers for each specific cancer. However, the cytotoxicity, genotoxicity, cellular fate, and cancer transcriptomics caused by bulk TiO_2-NPs are difficult to define in animal tissues, especially for comparisons between dissimilar tissues, such as liver, kidney, muscle, etc. Further investigation is required in this direction to unearth more evidence of TiO_2-NP distribution, biocompatibility, and low cytotoxicity in normal tissues. Overall, the fundamental understanding of the mechanical regulation of pristine TiO_2-NPs and doped-TiO_2-NPs with regard to cancer proteogenomics and chemical metabolomics is a dynamic process and is the basis for the development of future therapies.

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Abbreviations

8.

NPs	nanoparticles
nm	nanometer
μm	micrometer
TiO_2	titanium dioxide
Ag	silver
Zn	zinc

Cu	copper
Cr_2O_3	chromium (III) oxide
Mn_2O_3	manganese (III) oxide
Fe_2O_3	iron (III) oxide
NiO	nickel (II) oxide
CuO	copper (II) oxide
Gd	gadolinium
DOX	doxorubicin
Conc	concentrations
h	hours
mg/ml	milligram/milliliter
μg/L	microgram/Liter
TEM	transmission electron microscopy
FESEM	field emission scanning electron microscope
XRD	X-ray powder diffraction
Exp	experiment
Conc	concentrations
ROS	reactive oxygen species
H_2O_2	hydrogen peroxide
LC_{50}	50% lethal concentrations
D. rerio	Danio rerio
D. magna	Daphnia magna
SWCNT	single-walled carbon nanotubes
MWCNT	multi-walled CNT
NAD^+	nicotinamide adenine dinucleotide
$NADP^+$	nicotinamide adenine dinucleotide phosphate
DNA	deoxyribonucleic acid
RNA	ribonucleic acid
OS	oxidative stress

SOD	superoxide dismutase
TNF	tumor necrosis factor
MTT	methylthiazolyldiphenyl-tetrazolium bromide
LDH	lactate dehydrogenase
IL	interleukin
PCR	polymerase chain reaction
Ca^{2+}	calcium ions
PRDX	peroxiredoxins
MCP	monocyte chemoattractant protein
FDS	2′-deoxy-2′-(18F) fluoro-D-glucose
FITC	fluorescein isothiocyanate
MMP	mitochondrial membrane permeabilization
ATP	adenosine triphosphate
TCA	tricarboxylic acid cycle

9. References

1. Weir A, Westerhoff P, Fabricius L, Hristovski K, von Goetz N. Titanium dioxide nanoparticles in food and personal care products. Environ Sci Technol. 2012; 46: 2242–50. [PubMed: 22260395]
2. Maier T, Korting HC. Sunscreens - which and what for? Skin Pharmacol Physiol. 2005; 18: 253–262. [PubMed: 16113595]
3. Tucci P, Porta G, Agostini M, Dinsdale D, Iavicoli I, Cain K, et al. Metabolic effects of TiO2 nanoparticles, a common component of sunscreens and cosmetics, on human keratinocytes. Cell Death Dis. 2013; 4: e549.
4. Buser D, Broggini N, Wieland M, Schenk RK, Denzer AJ, Cochran DL, et al. Enhanced bone apposition to a chemically modified SLA titanium surface. J Dent Res. 2004; 83: 529–33. [PubMed: 15218041]
5. Schwarz F, Ferrari D, Herten M, Mihatovic I, Wieland M, Sager M, et al. Effects of surface hydrophilicity and microtopography on early stages of soft and hard tissue integration at non-submerged titanium implants: an immunohistochemical study in dogs. J Periodontol. 2007; 78: 2171–84. [PubMed: 17970685]
6. Baggs RB, Ferin J, Oberdorster G. Regression of pulmonary lesions produced by inhaled titanium dioxide in rats. Vet Pathol. 1997; 34: 592–7. [PubMed: 9396140]
7. Warheit DB, Hansen JF, Yuen IS, Kelly DP, Snajdr SI, Hartsky MA. Inhalation of high concentrations of low toxicity dusts in rats results in impaired pulmonary clearance mechanisms and persistent inflammation. Toxicol Appl Pharmacol. 1997; 145: 10–22. [PubMed: 9221819]
8. Ferin J, Oberdorster G. Biological effects and toxicity assessment of titanium dioxides: anatase and rutile. Am Ind Hyg Assoc J. 1985; 46: 69–72. [PubMed: 3976497]
9. Sang L, Zhao Y, Burda C. TiO2 nanoparticles as functional building blocks. Chem Rev. 2014; 114: 9283–318. [PubMed: 25294395]
10. Shevlin SA, Woodley SM. Electronic and Optical Properties of Doped and Undoped (TiO2)(n) Nanoparticles. J Phys Chem C. 2010; 114: 17333–43.

11. Lee KP, Trochimowicz HJ, Reinhardt CF. Pulmonary response of rats exposed to titanium dioxide (TiO2) by inhalation for two years. Toxicol Appl Pharmacol. 1985; 79: 179–92. [PubMed: 4002222]
12. Serpone N. Is the band gap of pristine TiO(2) narrowed by anion- and cation-doping of titanium dioxide in second-generation photocatalysts? J Phys Chem B. 2006; 110: 24287–93. [PubMed: 17134177]
13. Chen JS, Tan YL, Li CM, Cheah YL, Luan D, Madhavi S, et al. Constructing hierarchical spheres from large ultrathin anatase TiO2 nanosheets with nearly 100% exposed (001) facets for fast reversible lithium storage. J Am Chem Soc. 2010; 132: 6124–30. [PubMed: 20392065]
14. Dette C, Perez-Osorio MA, Kley CS, Punke P, Patrick CE, Jacobson P, et al. TiO2 anatase with a bandgap in the visible region. Nano Lett. 2014; 14: 6533–8. [PubMed: 25252265]
15. Tong T, Wilke CM, Wu J, Binh CT, Kelly JJ, Gaillard JF, et al. Combined Toxicity of Nano-ZnO and Nano-TiO2: From Single- to Multinanomaterial Systems. Environ Sci Technol. 2015; 49: 8113–23. [PubMed: 26070110]
16. Dambournet D, Belharouak I, Amine K. Tailored Preparation Methods of TiO2 Anatase, Rutile, Brookite: Mechanism of Formation and Electrochemical Properties. Chem Mater. 2010; 22: 1173–9.
17. Sanchez-Garcia MA, Bokhimi X, Maldonado-Alvarez A, Jimenez-Gonzalez AE. Effect of Anatase Synthesis on the Performance of Dye-Sensitized Solar Cells. Nanoscale Res Lett. 2015; 10: 991. [PubMed: 26220107]
18. Schottle C, Doronkin DE, Popescu R, Gerthsen D, Grunwaldt JD, Feldmann C. Ti(0) nanoparticles via lithium-naphthalenide-driven reduction. Chem Commun (Camb). 2016; 52: 6316–9. [PubMed: 27086750]
19. Moosavi MA, Sharifi M, Ghafary SM, Mohammadalipour Z, Khataee A, Rahmati M, et al. Photodynamic N-TiO2 Nanoparticle Treatment Induces Controlled ROS-mediated Autophagy and Terminal Differentiation of Leukemia Cells. Sci Rep. 2016; 6: 34413. [PubMed: 27698385]
20. Katsumata K, Ohno Y, Tomita K, Taniguchi T, Matsushita N, Okada K. Synthesis of amphiphilic brookite nanoparticles with high photocatalytic performance for wide range of application. ACS Appl Mater Interfaces. 2012; 4: 4846–52. [PubMed: 22860713]
21. Ahamed M, Khan MAM, Akhtar MJ, Alhadlaq HA, Alshamsan A. Ag-doping regulates the cytotoxicity of TiO2 nanoparticles via oxidative stress in human cancer cells. Sci Rep. 2017; 7: 17662. [PubMed: 29247182]
22. Chae SY, Park MK, Lee SK, Kim TY, Kim SK, Lee WI. Preparation of size-controlled TiO2 nanoparticles and derivation of optically transparent photocatalytic films. Chem Mater. 2003; 15: 3326–31.
23. Gindri IM, Frizzo CP, Bender CR, Tier AZ, Martins MA, Villetti MA, et al. Preparation of TiO(2) nanoparticles coated with ionic liquids: a supramolecular approach. ACS Appl Mater Interfaces. 2014; 6: 11536–43. [PubMed: 24933673]
24. Frank O, Zukalova M, Laskova B, Kurti J, Koltai J, Kavan L, Raman spectra of titanium dioxide (anatase, rutile) with identified oxygen isotopes (16, 17, 18), Phys Chem Chem Phys. 2012; 14(42): 14567–72. [PubMed: 23014450]
25. Naumenko D, Snitka V, Snopok B, Arpiainen S, Lipsanen H, Graphene-enhanced Raman imaging of TiO2 nanoparticles, Nanotechnology. 2012; 23(46): 465703.
26. Kamps K, Leek R, Luebke L, Price R, Nelson M, Simonet S, Eggert DJ, Atesin TA, Brown EM, Surface modification of the TiO2 nanoparticle surface enables fluorescence monitoring of aggregation and enhanced photoreactivity, Integr Biol (Camb). 2013; 5(1): 133–43. [PubMed: 22968372]
27. Ma H, Brennan A, Diamond SA, Photocatalytic reactive oxygen species production and phototoxicity of titanium dioxide nanoparticles are dependent on the solar ultraviolet radiation spectrum, Environ Toxicol Chem. 2012; 31(9): 2099–107. [PubMed: 22707245]
28. Marslin G, Sheeba CJ, Franklin G, Nanoparticles Alter Secondary Metabolism in Plants via ROS Burst, Front Plant Sci. 2017; 8: 832. [PubMed: 28580002]
29. Kim C, Kim S, Oh WK, Choi M, Jang J. Efficient intracellular delivery of camptothecin by silica/titania hollow nanoparticles. Chemistry. 2012; 18: 4902–8. [PubMed: 22422377]

30. Wang T, Jiang H, Wan L, Zhao Q, Jiang T, Wang B, et al. Potential application of functional porous TiO2 nanoparticles in light-controlled drug release and targeted drug delivery. Acta Biomater. 2015; 13: 354–63. [PubMed: 25462846]
31. Lovern SB, Klaper R. Daphnia magna mortality when exposed to titanium dioxide and fullerene (C60) nanoparticles. Environ Toxicol Chem. 2006; 25: 1132–7. [PubMed: 16629153]
32. Lee KP, Henry NW 3rd, Trochimowicz HJ, Reinhardt CF. Pulmonary response to impaired lung clearance in rats following excessive TiO2 dust deposition. Environ Res. 1986; 41: 144–67. [PubMed: 3757966]
33. Bernard BK, Osheroff MR, Hofmann A, Mennear JH. Toxicology and carcinogenesis studies of dietary titanium dioxide-coated mica in male and female Fischer 344 rats. J Toxicol Environ Health. 1990; 29: 417–29. [PubMed: 2325155]
34. Redline S, Barna BP, Tomashefski JF Jr., Abraham JL. Granulomatous disease associated with pulmonary deposition of titanium. Br J Ind Med. 1986; 43: 652–6. [PubMed: 3778834]
35. Geiser M, Casaulta M, Kupferschmid B, Schulz H, Semmler-Behnke M, Kreyling W, The role of macrophages in the clearance of inhaled ultrafine titanium dioxide particles, Am J Respir Cell Mol Biol. 2008; 38(3): 371–6. [PubMed: 17947511]
36. Bhattacharya K, Davoren M, Boertz J, Schins RP, Hoffmann E, Dopp E, Titanium dioxide nanoparticles induce oxidative stress and DNA-adduct formation but not DNA-breakage in human lung cells, Part Fibre Toxicol. 2009; 6: 17. [PubMed: 19545397]
37. Wang JJ, Sanderson BJ, Wang H, Cyto- and genotoxicity of ultrafine TiO2 particles in cultured human lymphoblastoid cells, Mutat Res. 2007; 628(2): 99–106. [PubMed: 17223607]
38. Lindenschmidt RC, Driscoll KE, Perkins MA, Higgins JM, Maurer JK, Belfiore KA. The comparison of a fibrogenic and two nonfibrogenic dusts by bronchoalveolar lavage. Toxicol Appl Pharmacol. 1990; 102: 268–81. [PubMed: 2154066]
39. Humans IWGotEoCRt. Inorganic and organic lead compounds. IARC Monogr Eval Carcinog Risks Hum. 2006; 87: 1–471. [PubMed: 17191367]
40. Borm PJ, Schins RP, Albrecht C. Inhaled particles and lung cancer, part B: paradigms and risk assessment. Int J Cancer. 2004; 110: 3–14. [PubMed: 15054863]
41. Dankovic D, Kuempel E, Wheeler M. An approach to risk assessment for TiO2. Inhal Toxicol. 2007; 19 Suppl 1: 205–12. [PubMed: 17886069]
42. Hong F, Wang L, Yu X, Zhou Y, Hong J, Sheng L, Toxicological effect of TiO2 nanoparticle-induced myocarditis in mice, Nanoscale Res Lett 10(1) (2015) 1029. [PubMed: 26269254]
43. Yang SP, Bar-Ilan O, Peterson RE, Heideman W, Hamers RJ, Pedersen JA. Influence of humic acid on titanium dioxide nanoparticle toxicity to developing zebrafish. Environ Sci Technol. 2013; 47: 4718–25. [PubMed: 23347333]
44. Hanot-Roy M, Tubeuf E, Guilbert A, Bado-Nilles A, Vigneron P, Trouiller B, et al. Oxidative stress pathways involved in cytotoxicity and genotoxicity of titanium dioxide (TiO2) nanoparticles on cells constitutive of alveolo-capillary barrier in vitro. Toxicol In Vitro. 2016; 33: 125–35. [PubMed: 26928046]
45. Faria M, Navas JM, Raldua D, Soares AM, Barata C. Oxidative stress effects of titanium dioxide nanoparticle aggregates in zebrafish embryos. Sci Total Environ. 2014; 470–471: 379–89.
46. Henkel CV, Dirks RP, Jansen HJ, Forlenza M, Wiegertjes GF, Howe K, et al. Comparison of the Exomes of Common Carp (Cyprinus carpio) and Zebrafish (Danio rerio). Zebrafish. 2012; 9: 59–67. [PubMed: 22715948]
47. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, et al. The zebrafish reference genome sequence and its relationship to the human genome. Nature. 2013; 496: 498–503. [PubMed: 23594743]
48. Church DM, Schneider VA, Graves T, Auger K, Cunningham F, Bouk N, et al. Modernizing Reference Genome Assemblies. Plos Biology. 2011; 9.
49. Kuklina I, Kouba A, Kozak P. Real-time monitoring of water quality using fish and crayfish as bio-indicators: a review. Environmental Monitoring and Assessment. 2013; 185: 5043–53. [PubMed: 23054288]

50. Tan FX, Wang M, Wang WM, Lu YN. Comparative evaluation of the cytotoxicity sensitivity of six fish cell lines to four heavy metals in vitro. Toxicology in Vitro. 2008; 22: 164–70. [PubMed: 17931828]
51. Federici G, Shaw BJ, Handy RD. Toxicity of titanium dioxide nanoparticles to rainbow trout (Oncorhynchus mykiss): gill injury, oxidative stress, and other physiological effects. Aquat Toxicol. 2007; 84: 415–30. [PubMed: 17727975]
52. Delmond KA, Vicari T, Guiloski IC, Dagostim AC, Voigt CL, Silva de Assis HC, Ramsdorf WA, Cestari MM, Antioxidant imbalance and genotoxicity detected in fish induced by titanium dioxide nanoparticles (NpTiO2) and inorganic lead (PbII), Environ Toxicol Pharmacol. 2019; 67: 42–52. [PubMed: 30711874]
53. Ramsden CS, Smith TJ, Shaw BJ, Handy RD. Dietary exposure to titanium dioxide nanoparticles in rainbow trout, (Oncorhynchus mykiss): no effect on growth, but subtle biochemical disturbances in the brain. Ecotoxicology. 2009; 18: 939–51. [PubMed: 19590957]
54. Zhu X, Chang Y, Chen Y. Toxicity and bioaccumulation of TiO2 nanoparticle aggregates in Daphnia magna. Chemosphere. 2010; 78: 209–15. [PubMed: 19963236]
55. Ma H, Brennan A, Diamond SA. Phototoxicity of TiO2 nanoparticles under solar radiation to two aquatic species: Daphnia magna and Japanese medaka. Environ Toxicol Chem. 2012; 31: 1621–9. [PubMed: 22544710]
56. Mansfield CM, Alloy MM, Hamilton J, Verbeck GF, Newton K, Klaine SJ, et al. Photo-induced toxicity of titanium dioxide nanoparticles to Daphnia magna under natural sunlight. Chemosphere. 2015; 120: 206–10. [PubMed: 25062026]
57. Zhu XS, Zhu L, Chen YS, Tian SY. Acute toxicities of six manufactured nanomaterial suspensions to Daphnia magna. Journal of Nanoparticle Research. 2009; 11: 67–75.
58. Amiano I, Olabarrieta J, Vitorica J, Zorita S. Acute toxicity of nanosized TiO(2) to Daphnia magna under UVA irradiation. Environ Toxicol Chem. 2012; 31: 2564–6. [PubMed: 22887344]
59. Wiench K, Wohlleben W, Hisgen V, Radke K, Salinas E, Zok S, et al. Acute and chronic effects of nano- and non-nano-scale TiO(2) and ZnO particles on mobility and reproduction of the freshwater invertebrate Daphnia magna. Chemosphere. 2009; 76: 1356–65. [PubMed: 19580988]
60. Wang J, Zhu X, Zhang X, Zhao Z, Liu H, George R, et al. Disruption of zebrafish (Danio rerio) reproduction upon chronic exposure to TiO(2) nanoparticles. Chemosphere. 2011; 83: 461–7. [PubMed: 21239038]
61. Park HG, Yeo MK. Comparison of gene expression changes induced by exposure to Ag, Cu-TiO2, and TiO2 nanoparticles in zebrafish embryos. Mol Cell Toxicol. 2013; 9: 129–39.
62. Kim MS, Louis KM, Pedersen JA, Hamers RJ, Peterson RE, Heideman W. Using citrate-functionalized TiO2 nanoparticles to study the effect of particle size on zebrafish embryo toxicity. Analyst. 2014; 139: 964–72. [PubMed: 24384696]
63. Diniz MS, de Matos AP, Lourenco J, Castro L, Peres I, Mendonca E, et al. Liver alterations in two freshwater fish species (Carassius auratus and Danio rerio) following exposure to different TiO(2) nanoparticle concentrations. Microsc Microanal. 2013; 19: 1131–40. [PubMed: 23931156]
64. Nowack B, Bucheli TD. Occurrence, behavior and effects of nanoparticles in the environment. Environ Pollut. 2007; 150: 5–22. [PubMed: 17658673]
65. Chen X, Mao SS. Titanium dioxide nanomaterials: synthesis, properties, modifications, and applications. Chem Rev. 2007; 107: 2891–959. [PubMed: 17590053]
66. Menard A, Drobne D, Jemec A. Ecotoxicity of nanosized TiO2. Review of in vivo data. Environ Pollut. 2011; 159: 677–84. [PubMed: 21186069]
67. Kim TH, Shin SW, Park JS, Park CS. Genome wide identification and expressionprofile in epithelial cells exposed to TiO(2) particles. Environ Toxicol. 2015; 30: 293–300. [PubMed: 24023007]
68. Griffitt RJ, Luo J, Gao J, Bonzongo JC, Barber DS. Effects of particle composition and species on toxicity of metallic nanomaterials in aquatic organisms. Environ Toxicol Chem. 2008; 27: 1972–8. [PubMed: 18690762]
69. Reeves JF, Davies SJ, Dodd NJ, Jha AN. Hydroxyl radicals (*OH) are associated with titanium dioxide (TiO(2)) nanoparticle-induced cytotoxicity and oxidative DNA damage in fish cells. Mutat Res. 2008; 640: 113–22. [PubMed: 18258270]

70. Vevers WF, Jha AN. Genotoxic and cytotoxic potential of titanium dioxide (TiO2) nanoparticles on fish cells in vitro. Ecotoxicology. 2008; 17: 410–20. [PubMed: 18491228]
71. Farkas J, Peter H, Ciesielski TM, Thomas KV, Sommaruga R, Salvenmoser W, et al. Impact of TiO(2) nanoparticles on freshwater bacteria from three Swedish lakes. Sci Total Environ. 2015; 535: 85–93. [PubMed: 25813090]
72. Behzadi S, Serpooshan V, Tao W, Hamaly MA, Alkawareek MY, Dreaden EC, Brown D, Alkilany AM, Farokhzad OC, Mahmoudi M, Cellular uptake of nanoparticles: journey inside the cell, Chem Soc Rev. 2017; 46(14): 4218–4244. [PubMed: 28585944]
73. Lu PJ, Fang SW, Cheng WL, Huang SC, Huang MC, Cheng HF. Characterization of titanium dioxide and zinc oxide nanoparticles in sunscreen powder by comparing different measurement methods. J Food Drug Anal. 2018; 26: 1192–200. [PubMed: 29976411]
74. Lucky SS, Soo KC, Zhang Y. Nanoparticles in photodynamic therapy. Chem Rev.2015; 115: 1990–2042. [PubMed: 25602130]
75. Sakhrani NM, Padh H. Organelle targeting: third level of drug targeting. Drug Des Devel Ther. 2013; 7: 585–99.
76. Hou XS, Wang HS, Mugaka BP, Yang GJ, Ding Y. Mitochondria: promising organelle targets for cancer diagnosis and treatment. Biomater Sci. 2018; 6: 2786–97. [PubMed: 30182102]
77. Asharani PV, Hande MP, Valiyaveettil S. Anti-proliferative activity of silver nanoparticles. BMC Cell Biol. 2009; 10: 65. [PubMed: 19761582]
78. Bergin IL, Witzmann FA. Nanoparticle toxicity by the gastrointestinal route: evidence and knowledge gaps. Int J Biomed Nanosci Nanotechnol. 2013; 3.
79. Gupta BN, Mathur AK. Toxicity of heavy metals (a review). Indian J Med Sci. 1983; 37: 236–40. [PubMed: 6370856]
80. Marano F, Hussain S, Rodrigues-Lima F, Baeza-Squiban A, Boland S. Nanoparticles: molecular targets and cell signalling. Arch Toxicol. 2011; 85: 733–41. [PubMed: 20502881]
81. Rauch J, Kolch W, Laurent S, Mahmoudi M. Big signals from small particles: regulation of cell signaling pathways by nanoparticles. Chem Rev. 2013; 113: 3391–406. [PubMed: 23428231]
82. Ursini CL, Cavallo D, Fresegna AM, Ciervo A, Maiello R, Buresti G, Casciardi S, Tombolini F, Bellucci S, Iavicoli S, Comparative cyto-genotoxicity assessment of functionalized and pristine multiwalled carbon nanotubes on human lung epithelial cells, Toxicol In Vitro. 2012; 26(6): 831–40. [PubMed: 22640919]
83. Scherbart AM, Langer J, Bushmelev A, van Berlo D, Haberzettl P, van Schooten FJ, et al. Contrasting macrophage activation by fine and ultrafine titanium dioxide particles is associated with different uptake mechanisms. Part Fibre Toxicol. 2011; 8: 31. [PubMed: 21995556]
84. Ghosh M, Chakraborty A, Mukherjee A. Cytotoxic, genotoxic and the hemolytic effect of titanium dioxide (TiO2) nanoparticles on human erythrocyte and lymphocyte cells in vitro. J Appl Toxicol. 2013; 33: 1097–110. [PubMed: 23616399]
85. Elsabahy M, Wooley KL. Cytokines as biomarkers of nanoparticle immunotoxicity. Chem Soc Rev. 2013; 42: 5552–76. [PubMed: 23549679]
86. Armand L, Tarantini A, Beal D, Biola-Clier M, Bobyk L, Sorieul S, Pernet-Gallay K, Marie-Desvergne C, Lynch I, Herlin-Boime N, Carriere M, Long-term exposure of A549 cells to titanium dioxide nanoparticles induces DNA damage and sensitizes cells towards genotoxic agents, Nanotoxicology. 2016; 10(7): 913–23. [PubMed: 26785166]
87. Carocci A, Catalano A, Lauria G, Sinicropi MS, Genchi G. Lead Toxicity,Antioxidant Defense and Environment. Rev Environ Contam Toxicol. 2016; 238: 45–67. [PubMed: 26670034]
88. Zhu Y, Eaton JW, Li C, Titanium dioxide (TiO2) nanoparticles preferentiallyinduce cell death in transformed cells in a Bak/Bax-independent fashion, PLoS One. 2012; 7(11): e50607.
89. Magrez A, Horvath L, Smajda R, Salicio V, Pasquier N, Forro L, et al. Cellular toxicity of TiO2-based nanofilaments. ACS Nano. 2009; 3: 2274–80. [PubMed: 19610603]
90. Yuan YG, Gurunathan S. Combination of graphene oxide-silver nanoparticle nanocomposites and cisplatin enhances apoptosis and autophagy in human cervical cancer cells. Int J Nanomedicine. 2017; 12: 6537–58. [PubMed: 28919753]
91. Chen T, Yan J, Li Y, Genotoxicity of titanium dioxide nanoparticles, J Food Drug Anal. 2014; 22(1): 95–104. [PubMed: 24673907]

92. Rahmani Kukia N, Rasmi Y, Abbasi A, Koshoridze N, Shirpoor A, Burjanadze G, Saboory E, Bio-Effects of TiO2 Nanoparticles on Human Colorectal Cancer and Umbilical Vein Endothelial Cell Lines, Asian Pac J Cancer Prev 19(10) (2018) 2821–2829. [PubMed: 30361551]

93. Gurr JR, Wang AS, Chen CH, Jan KY. Ultrafine titanium dioxide particles in the absence of photoactivation can induce oxidative damage to human bronchial epithelial cells. Toxicology. 2005; 213: 66–73. [PubMed: 15970370]

94. Kang SJ, Kim BM, Lee YJ, Chung HW. Titanium dioxide nanoparticles trigger p53-mediated damage response in peripheral blood lymphocytes. Environ Mol Mutagen. 2008; 49: 399–405. [PubMed: 18418868]

95. Biola-Clier M, Beal D, Caillat S, Libert S, Armand L, Herlin-Boime N, Sauvaigo S, Douki T, Carriere M, Comparison of the DNA damage response in BEAS-2B and A549 cells exposed to titanium dioxide nanoparticles, Mutagenesis 32(1) (2017) 161–172. [PubMed: 27803034]

96. Val S, Hussain S, Boland S, Hamel R, Baeza-Squiban A, Marano F. Carbon black and titanium dioxide nanoparticles induce pro-inflammatory responses in bronchial epithelial cells: need for multiparametric evaluation due to adsorption artifacts. Inhal Toxicol. 2009; 21 Suppl 1: 115–22. [PubMed: 19558243]

97. Knaapen AM, Borm PJ, Albrecht C, Schins RP. Inhaled particles and lung cancer. Part A: Mechanisms. Int J Cancer. 2004; 109: 799–809. [PubMed: 15027112]

98. Jin CY, Zhu BS, Wang XF, Lu QH. Cytotoxicity of titanium dioxide nanoparticles in mouse fibroblast cells. Chem Res Toxicol. 2008; 21: 1871–7. [PubMed: 18680314]

99. Zhao J, Bowman L, Zhang X, Vallyathan V, Young SH, Castranova V, et al. Titanium dioxide (TiO2) nanoparticles induce JB6 cell apoptosis through activation of the caspase-8/Bid and mitochondrial pathways. J Toxicol Environ Health A. 2009; 72: 1141–9. [PubMed: 20077182]

100. Seta KA, Yuan Y, Spicer Z, Lu G, Bedard J, Ferguson TK, et al. The role of calcium in hypoxia-induced signal transduction and gene expression. Cell Calcium. 2004; 36: 331–40. [PubMed: 15261489]

101. Jones PA, Takai D. The role of DNA methylation in mammalian epigenetics. Science. 2001; 293: 1068–70. [PubMed: 11498573]

102. Fisichella M, Berenguer F, Steinmetz G, Auffan M, Rose J, Prat O, Intestinal toxicity evaluation of TiO2 degraded surface-treated nanoparticles: a combined physico-chemical and toxicogenomics approach in caco-2 cells, Part Fibre Toxicol. 2012; 9: 18. [PubMed: 22650444]

103. Gupta BN, Mathur AK. Toxicity of heavy metals (a review). Indian J Med Sci. 1983; 37: 236–40. [PubMed: 6370856]

104. Valko M, Morris H, Cronin MT. Metals, toxicity and oxidative stress. Curr Med Chem. 2005; 12: 1161–208. [PubMed: 15892631]

105. Ahamed M, Khan MA, Akhtar MJ, Alhadlaq HA, Alshamsan A. Role of Zn doping in oxidative stress mediated cytotoxicity of TiO2 nanoparticles in human breast cancer MCF-7 cells. Sci Rep. 2016; 6: 30196. [PubMed: 27444578]

106. Raja G, Kim S, Yoon D, Yoon C, Kim S. 1H NMR based metabolomics studies of the toxicity of titanium dioxide nanoparticles in zebrafish (Danio rerio). Bull. Korean Chem. Soc. 2018; 39: 33–39

107. Scherbart AM, Langer J, Bushmelev A, van Berlo D, Haberzettl P, van Schooten FJ, et al. Contrasting macrophage activation by fine and ultrafine titanium dioxide particles is associated with different uptake mechanisms. Part Fibre Toxicol. 2011; 8: 31. [PubMed: 21995556]

108. Ghosh M, Chakraborty A, Mukherjee A. Cytotoxic, genotoxic and the hemolytic effect of titanium dioxide (TiO2) nanoparticles on human erythrocyte and lymphocyte cells in vitro. J Appl Toxicol. 2013; 33: 1097–110. [PubMed: 23616399]

109. Kang K, Jung H, Lim JS. Cell Death by Polyvinylpyrrolidine-Coated Silver Nanoparticles is Mediated by ROS-Dependent Signaling. Biomol Ther (Seoul). 2012; 20: 399–405. [PubMed: 24009827]

110. Rajh T, Dimitrijevic NM, Bissonnette M, Koritarov T, Konda V. Titanium dioxide in the service of the biomedical revolution. Chem Rev. 2014; 114: 10177–216. [PubMed: 25171650]

111. Shi Y, Wang F, He J, Yadav S, Wang H. Titanium dioxide nanoparticles cause apoptosis in BEAS-2B cells through the caspase 8/t-Bid-independent mitochondrial pathway. Toxicol Lett. 2010; 196: 21–7. [PubMed: 20362650]
112. Shi H, Magaye R, Castranova V, Zhao J. Titanium dioxide nanoparticles: a review of current toxicological data. Part Fibre Toxicol. 2013; 10: 15. [PubMed: 23587290]
113. Chusuei CC, Wu CH, Mallavarapu S, Hou FY, Hsu CM, Winiarz JG, et al. Cytotoxicity in the age of nano: the role of fourth period transition metal oxide nanoparticle physicochemical properties. Chem Biol Interact. 2013; 206: 319–26. [PubMed: 24120544]
114. Ghosh M, Bandyopadhyay M, Mukherjee A. Genotoxicity of titanium dioxide (TiO2) nanoparticles at two trophic levels: plant and human lymphocytes. Chemosphere. 2010; 81: 1253–62. [PubMed: 20884039]
115. Wang Y, Cui H, Zhou J, Li F, Wang J, Chen M, et al. Cytotoxicity, DNA damage, and apoptosis induced by titanium dioxide nanoparticles in human non-small cell lung cancer A549 cells. Environ Sci Pollut Res Int. 2015; 22: 5519–30. [PubMed: 25339530]
116. Shukla RK, Kumar A, Vallabani NV, Pandey AK, Dhawan A. Titanium dioxide nanoparticle-induced oxidative stress triggers DNA damage and hepatic injury in mice. Nanomedicine (Lond). 2014; 9: 1423–34. [PubMed: 24367968]
117. Zhou Z, Song J, Nie L, Chen X. Reactive oxygen species generating systems meeting challenges of photodynamic cancer therapy. Chem Soc Rev. 2016; 45: 6597–626. [PubMed: 27722328]
118. Czajka M, Sawicki K, Sikorska K, Popek S, Kruszewski M, Kapka-Skrzypczak L. Toxicity of titanium dioxide nanoparticles in central nervous system. Toxicol In Vitro. 2015; 29: 1042–52. [PubMed: 25900359]
119. Jugan ML, Barillet S, Simon-Deckers A, Herlin-Boime N, Sauvaigo S, Douki T, et al. Titanium dioxide nanoparticles exhibit genotoxicity and impair DNA repair activity in A549 cells. Nanotoxicology. 2012; 6: 501–13. [PubMed: 21995316]
120. Runa S, Khanal D, Kemp ML, Payne CK. TiO2 Nanoparticles Alter the Expression of Peroxiredoxin Antioxidant Genes. J Phys Chem C. 2016; 120: 20736–42.
121. Kotagiri N, Sudlow GP, Akers WJ, Achilefu S. Breaking the depth dependency of phototherapy with Cerenkov radiation and low-radiance-responsive nanophotosensitizers. Nat Nanotechnol. 2015; 10: 370–9. [PubMed: 25751304]
122. Ramkumar KM, Manjula C, Gnanakumar G, Kanjwal MA, Sekar TV, Paulmurugan R, et al. Oxidative stress-mediated cytotoxicity and apoptosis induction by TiO2 nanofibers in HeLa cells. Eur J Pharm Biopharm. 2012; 81: 324–33. [PubMed: 22446064]
123. Martin A, Sarkar A. Epithelial to Mesenchymal transition, eIF2alpha phosphorylation and Hsp70 expression enable greater tolerance in A549 cells to TiO2 over ZnO nanoparticles. Sci Rep. 2019; 9: 436. [PubMed: 30679528]
124. Tang T, Zhang Z, Zhu X, Toxic Effects of TiO(2) NPs on Zebrafish, Int J Environ Res Public Health. 2019; 16(4): 523.
125. Yeo MK, Kim HE. Gene expression in zebrafish embryos following exposure to TiO2 nanoparticles. Mol Cell Toxicol. 2010; 6: 97–104.
126. Hovhannisyan G, Aroutiounian R, Liehr T. Chromosomal composition of micronuclei in human leukocytes exposed to mitomycin C. J Histochem Cytochem. 2012; 60: 316–22. [PubMed: 22260997]
127. Engin AB, Nikitovic D, Neagu M, Henrich-Noack P, Docea AO, Shtilman MI, Golokhvast K, Tsatsakis AM, Mechanistic understanding of nanoparticles' interactions with extracellular matrix: the cell and immune system, Part Fibre Toxicol 14(1) (2017) 22. [PubMed: 28646905]
128. Dejana E. Endothelial cell-cell junctions: happy together. Nat Rev Mol Cell Biol. 2004; 5: 261–70. [PubMed: 15071551]
129. Gavard J, Gutkind JS. VEGF controls endothelial-cell permeability by promoting the beta-arrestin-dependent endocytosis of VE-cadherin. Nat Cell Biol. 2006; 8: 1223–34. [PubMed: 17060906]
130. Setyawati MI, Tay CY, Chia SL, Goh SL, Fang W, Neo MJ, et al. Titanium dioxide nanomaterials cause endothelial cell leakiness by disrupting the homophilic interaction of VE-cadherin. Nat Commun. 2013; 4: 1673. [PubMed: 23575677]

131. Kiss B, Biro T, Czifra G, Toth BI, Kertesz Z, Szikszai Z, et al. Investigation of micronized titanium dioxide penetration in human skin xenografts and its effect on cellular functions of human skin-derived cells. Exp Dermatol. 2008; 17: 659–67. [PubMed: 18312389]

132. Tee JK, Ng LY, Koh HY, Leong DT, Ho HK. Titanium Dioxide Nanoparticles Enhance Leakiness and Drug Permeability in Primary Human Hepatic Sinusoidal Endothelial Cells. Int J Mol Sci. 2018; 20.

133. Tiwari A, Addis Jones O, Chan KL. 53BP1 can limit sister-chromatid rupture and rearrangements driven by a distinct ultrafine DNA bridging-breakage process. Nat Commun. 2018; 9: 677. [PubMed: 29445165]

134. Barra V, Fachinetti D. The dark side of centromeres: types, causes and consequences of structural abnormalities implicating centromeric DNA. Nat Commun. 2018; 9: 4340. [PubMed: 30337534]

135. Vileno B, Lekka M, Sienkiewicz A, Jeney S, Stoessel G, Lekki J, et al. Stiffness alterations of single cells induced by UV in the presence of nanoTiO2. Environ Sci Technol. 2007; 41: 5149–53. [PubMed: 17711237]

136. Lai JC, Lai MB, Jandhyam S, Dukhande VV, Bhushan A, Daniels CK, et al. Exposure to titanium dioxide and other metallic oxide nanoparticles induces cytotoxicity on human neural cells and fibroblasts. Int J Nanomedicine. 2008; 3: 533–45. [PubMed: 19337421]

137. Patti GJ, Yanes O, Siuzdak G. Innovation: Metabolomics: the apogee of the omics trilogy. Nat Rev Mol Cell Biol. 2012; 13: 263–9. [PubMed: 22436749]

138. Fernie AR, Trethewey RN, Krotzky AJ, Willmitzer L. Metabolite profiling: from diagnostics to systems biology. Nat Rev Mol Cell Biol. 2004; 5: 763–9. [PubMed: 15340383]

139. Seibel BA, Walsh PJ. Trimethylamine oxide accumulation in marine animals: relationship to acylglycerol storage. J Exp Biol. 2002; 205: 297–306. [PubMed: 11854367]

140. Raja G, Kim S, Yoon D, Yoon C, Kim S. 1H-NMR-based metabolomics studies of the toxicity of mesoporous carbon nanoparticles in zebrafish (Danio rerio). Bull. Korean Chem. Soc. 2017; 38: 271–277.

141. Saugstad OD. Hypoxanthine as an indicator of hypoxia: its role in health and disease through free radical production. Pediatr Res. 1988; 23: 143–50. [PubMed: 3281119]

142. Gluick TC, Yadav S. Trimethylamine N-oxide stabilizes RNA tertiary structure and attenuates the denaturating effects of urea. J Am Chem Soc. 2003; 125: 4418–9. [PubMed: 12683801]

143. Kumar A, Pandey AK, Singh SS, Shanker R, Dhawan A. Engineered ZnO and TiO(2) nanoparticles induce oxidative stress and DNA damage leading to reduced viability of Escherichia coli. Free Radic Biol Med. 2011; 51: 1872–81. [PubMed: 21920432]

144. Caudill MA, Wang JC, Melnyk S, Pogribny IP, Jernigan S, Collins MD, et al. Intracellular S-adenosylhomocysteine concentrations predict global DNA hypomethylation in tissues of methyl-deficient cystathionine beta-synthase heterozygous mice. J Nutr. 2001; 131: 2811–8. [PubMed: 11694601]

145. Choy JS, Wei S, Lee JY, Tan S, Chu S, Lee TH. DNA methylation increases nucleosome compaction and rigidity. J Am Chem Soc. 2010; 132: 1782–3. [PubMed: 20095602]

146. Nomura W, Barbas CF 3rd. In vivo site-specific DNA methylation with a designed sequence-enabled DNA methylase. J Am Chem Soc. 2007; 129: 8676–7. [PubMed: 17583340]

147. Ganguly P, Boserman P, van der Vegt NFA, Shea JE, Trimethylamine N-oxide Counteracts Urea Denaturation by Inhibiting Protein-Urea Preferential Interaction, J Am Chem Soc. 2018; 140(1): 483–492. [PubMed: 29214802]

148. Ma J, Pazos IM, Gai F. Microscopic insights into the protein-stabilizing effect of trimethylamine N-oxide (TMAO). Proc Natl Acad Sci U S A. 2014; 111: 8476–81. [PubMed: 24912147]

149. Bo Y, Jin C, Liu Y, Yu W, Kang H. Metabolomic analysis on the toxicological effects of TiO(2) nanoparticles in mouse fibroblast cells: from the perspective of perturbations in amino acid metabolism. Toxicol Mech Methods. 2014; 24: 461–9. [PubMed: 24965839]

150. Kamkaew A, Chen F, Zhan Y, Majewski RL, Cai W. Scintillating Nanoparticles as Energy Mediators for Enhanced Photodynamic Therapy. ACS Nano. 2016; 10: 3918–35. [PubMed: 27043181]

151. Hu J, Tang Y, Elmenoufy AH, Xu H, Cheng Z, Yang X. Nanocomposite-Based Photodynamic Therapy Strategies for Deep Tumor Treatment. Small. 2015; 11: 5860–87. [PubMed: 26398119]

152. Long TC, Saleh N, Tilton RD, Lowry GV, Veronesi B. Titanium dioxide (P25) produces reactive oxygen species in immortalized brain microglia (BV2): implications for nanoparticle neurotoxicity. Environ Sci Technol. 2006; 40: 4346–52. [PubMed: 16903269]

153. Tripathy N, Kim DH, Metal oxide modified ZnO nanomaterials for biosensor applications, Nano Converg. 2018; 5(1): 27. [PubMed: 30467757]

154. Raja G, Jang YK, Suh JS, Prabhakaran VS, Kim TJ Advanced understanding of genetic risk and metabolite signatures in construction workers via cytogenetics and metabolomics analysis. Process Biochemistry. 2019;

155. Ren W, Iqbal MZ, Zeng L, Chen T, Pan Y, Zhao J, Yin H, Zhang L, Zhang J, Li A, Wu A. Black TiO2 based core-shell nanocomposites as doxorubicin carriers for thermal imaging guided synergistic therapy of breast cancer. Nanoscale. 2017; 9(31): 11195–11204. [PubMed: 28749498]

156. Mou J, Lin T, Huang F, Chen H, Shi J. Black titania-based theranostic nanoplatform for single NIR laser induced dual-modal imaging-guided PTT/PDT. Biomaterials. 2016; 84: 13–24. [PubMed: 26803408]

157. Wang S, Ren W, Wang J, Jiang Z, Saeed M, Zhang L, Li A, Wu A. Black TiO2-based nanoprobes for T1-weighted MRI-guided photothermal therapy in CD133 high expressed pancreatic cancer stem-like cells. Biomater Sci. 2018; 6(8): 2209–2218. [PubMed: 29947365]

158. Ren W, Yan Y, Zeng L, Shi Z, Gong A, Schaaf P, Wang D, Zhao J, Zou B, Yu H, Chen G, Brown EM, Wu A. A Near Infrared Light Triggered Hydrogenated Black TiO2 for Cancer Photothermal Therapy. Adv Healthc Mater. 2015; 4(10): 1526–36. [PubMed: 26010821]

159. Saeed M, Iqbal MZ, Ren WZ, Xia YZ, Liu C, Khan WS, Wu AG. Controllable synthesis of Fe3O4 nanoflowers: enhanced imaging guided cancer therapy and comparison of photothermal efficiency with black-TiO2. J Mater Chem B. 2018; 6(22): 3800–3810. [PubMed: 32254842]

160. Iqbal MZ, Ren WZ, Saeed M, Chen TX, Ma XH, Yu X, Zhang JC, Zhang LL, Li AG, Wu AG. A facile fabrication route for binary transition metal oxide-based Janus nanoparticles for cancer theranostic applications. Nano Res. 2018; 11(10): 5735–5750.

Highlights

- We present a comprehensive review of the microenvironmental influence of TiO2-NPs induced various mechanical stimuli on tumor cells.
- The mechanosensitive proteogenomics degradation, inflammatory responses by TiO2-NPs exposure are examined.
- The omics functional analysis shows gene mutations, protein alterations, and metabolite changes with TiO2-NPs exposures.
- Cancer proteogenomics and metabolomics by TiO2-NPs are dynamic platforms for cancer therapy.

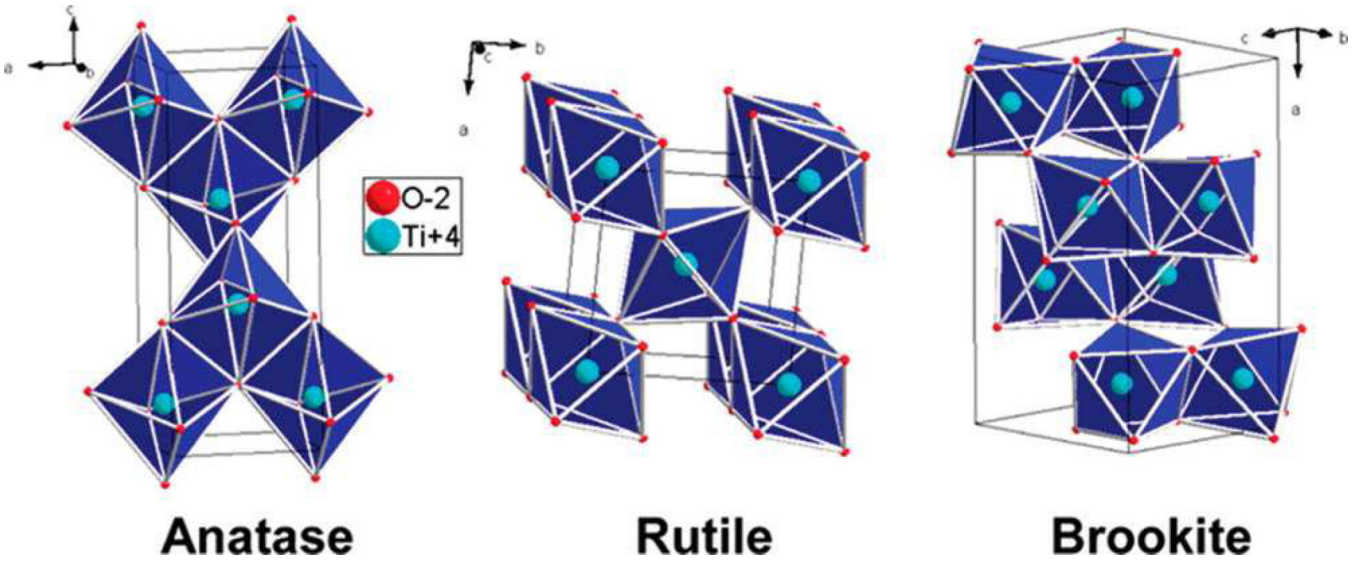

Figure 1.

The structural description of TiO_2 and its three different forms. Anatase (tetragonal, a = 3.785 Å, c = 9.513 Å), rutile (tetragonal, a = 4.593 Å, c = 2.959 Å), and brookite (orthorhombic, a = 9.181 Å, b = 5.455 Å, c = 5.142 Å). Reproduced with permission from ref 9, 16. Copyright 2010 American Chemical Society.

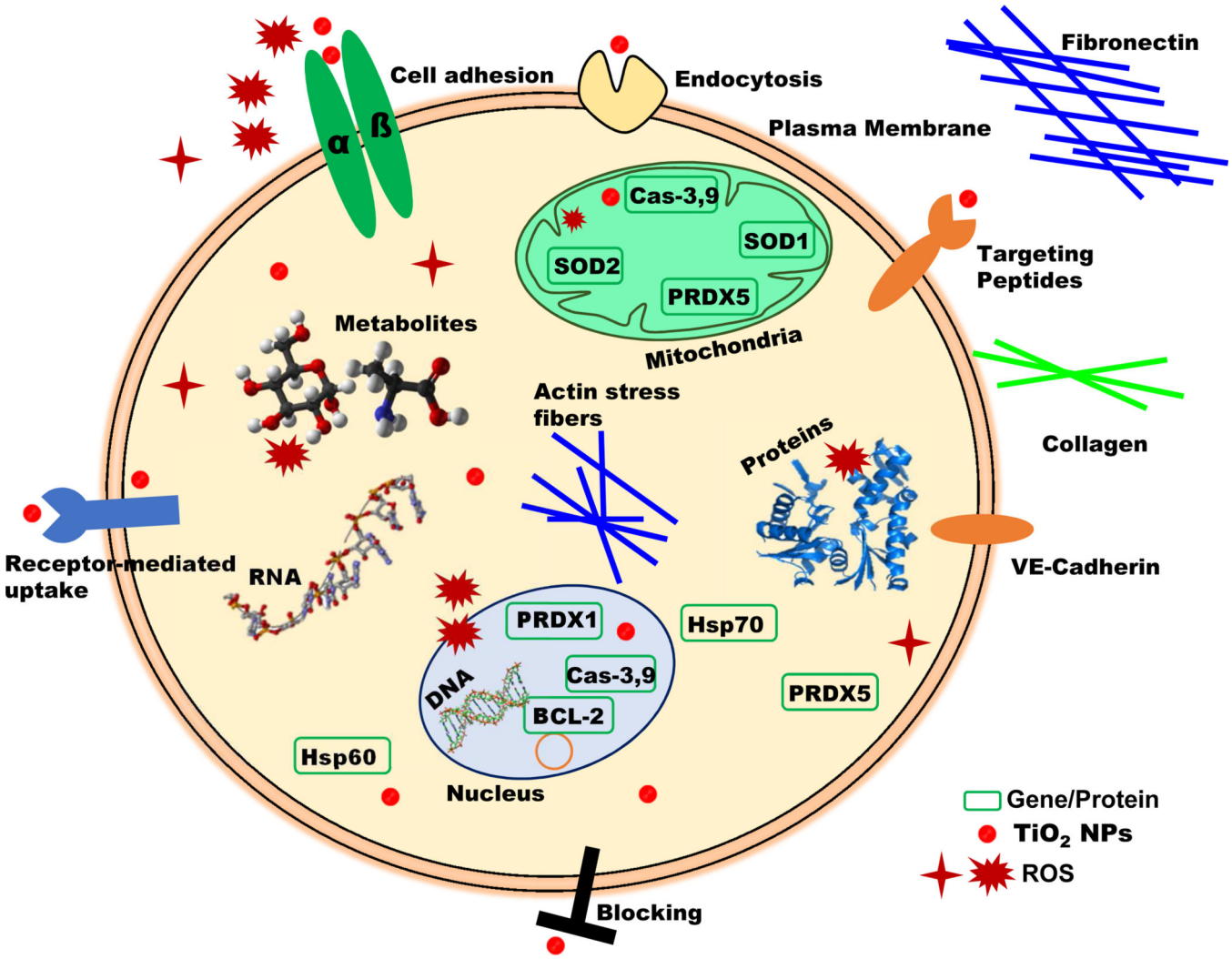

Figure 2.

Cellular uptake pathways, origins, and influence of TiO_2-NPs on tumor cellular microenvironment. Tumor heterogeneity involves the dysregulation of genes, proteins, and metabolites. TiO_2-NPs can block tumorigenesis and TiO_2-mediated activation of OS signaling. Several NP formulations on the cell surface or upon endocytosis were shown to trigger the production of ROS. TiO_2-NPs are depicted as red circles; ROS are shown as stars.

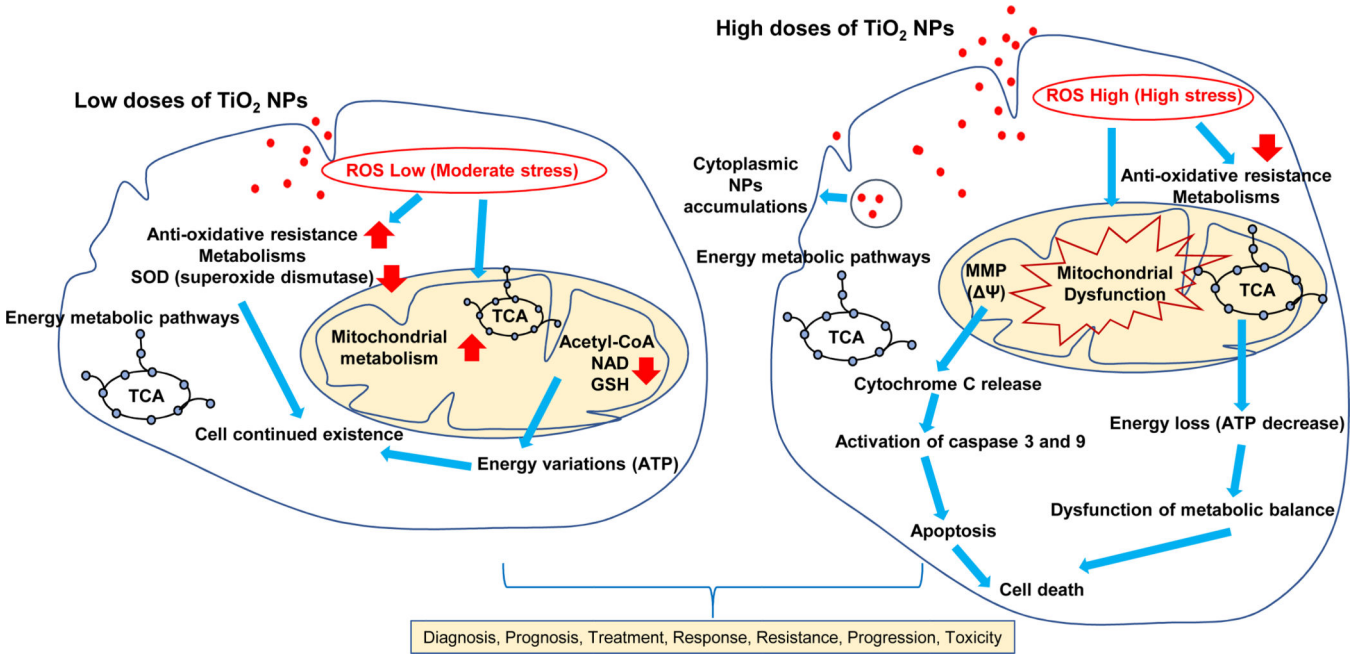

Figure 3.

In proliferating tumor cells, the schematic representation of TiO_2-NPs applied to complex environmental metabolism and catabolized through major oxidative stress, metabolites alteration, gene and protein dysregulation. This combination treatment with pure and functionalized TiO_2-NPs suppresses tumor growth through the influence of ROS.

Table 1

Uptake and bioaccumulation of different types of TiO_2-NPs in tissue-specific mechanobiological analysis.

NPs	Duration	NP concentrations	Organism	Mechanoregulation and Metabolic effects	Ref
P25 TiO_2-NPs	6 days	1 mg/mL	*Danio rerio embryos*	ROS production/oxidative stress	[45]
TiO_2-NPs	7, 14 days	0.1, 0.5, 1.0 mg/L	*Juvenile rainbow trout*	Respiratory toxicity and disturbances of trace elements such as Zn and Cu in metabolism	[51]
P25 TiO_2	0–4 days	0.1, 1.0, 5.0 mg/L	*Daphnia magna*	Low toxicity was found	[54,57]
P25 TiO_2	2 days 4 days	2 g/L 2 g/L	*Daphnia magna*	LC_{50} 29.8 μg/L	[55]
	4 days	2 g/L	*Japanese medaka fish larvae*	LC_{50} 2.2 mg/L	
Anatase TiO_2	8 h	20 & 200 mg/L	*Daphnia magna*	Significant mortality	[56]
P25 TiO_2	2 days	1–10.5 mg/L	*Daphnia magna*	Increased immobilization; increased toxicity with UVA	[58,59]
Anatase TiO_2	13 weeks	0.1, 1.0 mg/L	Adult zebrafish	Altered gene expression; 0.1 mg/L of $nTiO_2$ is toxic to the reproductive system;	[60]
Ag NPs; Cu-TiO_2- NPs; TiO_2-NPs	2, 5, 8, 22, 27, 32, 48 and 72 h	20 ppt	Adult zebrafish embryos	37 up and 33 downregulated immune response genes was measured by TiO_2; genotoxicity induced by these three NPs.	[61]
Citrate-TiO_2-NPs	24 h	0-1000 μg/ml	zebrafish (*Danio rerio*)	6 nm TiO_2-NPs were more potent in producing ROS; NPs surface area-based toxicity was discovered.	[62]
P25 TiO_2-NPs	14 days	0.01, 0.1, 1, 10, and 100 mg/L	*C. auratus and Danio rerio*	Fish liver metabolism affected; oxidative stress	[63]
Anatase TiO_2	2 h	1000 μ/mL	goldfish skin cells	Lowered cell viability	[68,69]
Rutile/anatase TiO_2-NPs	4 h-2 days	500 ng/mL	rainbow trout gonadal tissue	Cytotoxicity; DNA strand breaks	[70]
Anatase/rutile TiO_2 -NPs	5 days	15–1000 g/L	freshwater bacteria	UV/PAR did not enhance toxicity	[71]

Table 2.

Toxicokinetic findings and cytotoxicity and genotoxicity effects due to physicochemical properties of TiO_2-NP exposure in non-cancer and cancer cell lines.

Disease	Cell lines	Crystal types	Particle Size (nm)	Dose	Mechanoregulation and Metabolic effects	Ref
Hepatocellular carcinoma	Human liver cancer (HepG2)	Ag-TiO_2-NPs	0.353 nm	25, 50, 100 μg mL	TiO_2-NPs induced cyto- and genotoxicity in human liver cancer (HepG2) cells via oxidative stress	21
Carcinoma	A549	100% anatase TiO_2; 60% anatase TiO_2; 40% rutile mixture of TiO_2	10.1 ± 1.0 5.2 ± 0.34 3.2 ± 0.34	3 μg/mL- 3 mg/mL for 48 h	Cell viability; DNA-based genotoxicity; Mitochondrial activity; cellular redox status	86
Adenosquamous carcinoma	Human lung tumor	Na(x) $TiO_{2+\delta}$ or Hy TiO_2+ δ	12 nm nanotubes 75 nm nanowires	0.02–2 μg /mL	Nanofilaments impaired cell proliferation; Hy TiO_2+δ were more toxic than Na(x) TiO_2+δ forms; cell morphology was altered.	89
Colorectal carcinoma	HCT116	TiO_2-NPs powder	--	50,100, 200, 400 μg/mL	Cytotoxicity, cell viability, apoptosis regulators (p53, Bax, Bcl-2)	92
Acute monocytic leukemia	Human monocytes THP-1 cells A549	≥ 99 % TiO_2	70±20	0.1, 0.2, 0.5 μg/mL	Cell mortality; DNA activity; protein expression; DNA repair genes and pathways	95
Carcinoma	A549 human lung epithelial cells	TiO_2 -NPs	50 nm	0.25, 5, 40 μg/mL	Phagocytized TiO_2 into vacuoles (early) or lamellar bodies; epithelial cells TiO_2 externally connected with plasma membrane	103
Adenocarcinoma	human breast cancer (MCF-7)	Zn-TiO_2-NPs	0.352 nm	50,100, 200 μg /mL	Cytotoxicity and cell cycle arrest	104
Cervical carcinoma cells	HeLa	≥99.5% trace metals basis TiO_2	21 nm,	400, 270, and 160 μg/mL	Up or downregulation of peroxiredoxin-1 3 4 5; actin expression has confirmed	119
Carcinoma	A549	P25 Degussa TiO_2, 75% anatase CEA TiO_2, 95% anatase sigma TiO_2, 100% anatase sigma rutile TiO_2, 100% rutile	25±7 12±3 142±36 9±3	0.25–100 μg /mL	Cell membrane damage; decreases the cell capability; TiO_2-NP site in cytoplasm, nucleus	120
Normal	16HBE14o-	99.9% anatase TiO_2	15	0–160 μg/cm^2	Dose dependent cytotoxic effects; dose dependent increase in IL-6, TNF-α mRNA; increased intracellular cytoskeletal proteins; absorption of GM-CSF and TNF-α by TiO_2-NPs.	96
Normal	Human bronchial epithelial cells (BEAS-2B)	Anatase TiO_2 Rutile TiO_2	10; 20; 200; > 200	10 μg/mL	Induction of oxidative stress, ROS; hinders cellular development.	104
Normal	BEAS-2B	99.7% anatase TiO_2	< 25	0–100 μg/mL	Dose dependent reduction of cell viability; initiation of apoptosis; ROS can dependently increase based on TiO_2 dose.	111 112

Disease	Cell lines	Crystal types	Particle Size (nm)	Dose	Mechanoregulation and Metabolic effects	Ref
Normal	BS-C-1	≥99.5% trace metals basis	21 nm,	400, 270, and 160 μg/mL	Up or downregulation of peroxiredoxin family; β-actin expression was confirmed.	119
Normal	Endothelial cell	TiO_2	48–55 nm	0.4, 0.8, 4, 8 μg /mL	Disturbs cell–cell interactions, VE–cadherin may be affected and degraded	31 130
Normal	HaCaT cell line; Human immortalized sebaceous gland cell line (SZ95); Primary human melanocytes.	Anatase TiO_2	9	0.15–15 μg/cm^2	TiO_2 was spotted at perinuclear area and melanocyte cytoplasm; slow and adjustable rise in $[Ca^{2+}]$ in fibroblasts and melanocytes.	131
Normal	Normal human skin fibroblasts (CCL-110)	Anatase TiO_2	5	4 μg /ml	Cell stiffness decreased; beta-carotene prevented photo-oxidative stiffness alterations	135
Normal Likely glioblastoma	Human fibroblasts (HFF1); Human astrocytoma U87 cells;	Rutile TiO_2 99.7% anatase TiO_2	< 25	0.1–100 μg/mL	Micro and nano TiO_2 particles caused a reduction of cell viability in U87 and HFF1 cells	136
Normal	Immortalized mouse brain microglia (BV2)	Degussa P25 TiO_2 70% anatase; 30% rutile	~ 30	2.5–120 mg/kg	Rapid and constant release of H_2O_2 and O_2; small clusters phagocytosed and internalized into the cytoplasm.	152

Table 3.

Mechanoregulation of pure and doped-TiO_2-NPs in specific gene and protein functions in tumor microenvironment after mechanistic stress by external forces

Gene/Protein	Materials type (nm)	Structure	Distribution	Mechanobiological functions	Ref
IL-8; 53BP1 foci	100% anatase TiO_2; 60% anatase TiO_2; 40% rutile mixture of TiO_2	Monomers	Macrophages; endothelial cells	Cytokine activity; genotoxicity;	86
IL-1 beta	TiO_2-NPs	Tetrahedron	Macrophages; endothelial cells	Cytokine activity; immune regulation responses;	95
SOD1	Zn-TiO_2-NPs; TiO_2	Homodimer; Non-disulfide linked	Cytosol	Familial amyotrophic lateral sclerosis (ALS) by SOD1 mutation	104, 118, 130
SOD2	Zn-TiO_2-NPs	Tetramer	Mitochondria	Protection of mitochondria from ROS damage	104, 118
GPx; CPD-64; 8oxo; CisP; Glycol	TiO_2-A12; A25; A140; R68; R20	--	Nucleus;	DNA repair ability; oxidative DNA damage; genotoxic stress	119
PRDX1; PRDX3; PRDX4; PRDX5	TiO_2-NP	Dimer	Cytosol; Nucleus; Mitochondria; ER; extracellular;	Signal regulation/transduction; apoptosis; antioxidant activity; ER folds; unknown	120
Hsp 60	TiO_2-PEG; TiO_2-Tf; Tc-Tf; and TiO_2-Tf-Tc	Monomers	Mitochondria	Protein folding and assembly; tumor-targeting mechanisms	121
Hsp70; Bax; Bcl- 2; Cyt-C; Cleaved caspase-3	TiO_2 nanofibers; TiO_2-NM	Tetramer	Cytosol/ Nucleus/ Mitochondria	Prevention of aggregation of unfolded protein chains; apoptosis related protein;	122,130
E-Cadherin; N-Cadherin; EGFR	TiO_2-NM	Homodimer	Plasma membrane	Reponses to hypoxia; B cell homeostasis; proteolysis	122, 123,130
Bcl-2, Bax, Cyt-C, β-actin; Dsg-1	TiO_2 nanofibers; Anatase TiO_2	Tetramer	Nuclear Membrane; nucleoplasm	Cell morphogenesis; ossification; apoptosis; Ca^{2+} imaging	122, 131
VE-cadherin; Caspase 3, 8, 9; SOD1	TiO_2-NM	Homodimer	Plasma membrane	Calcium ion binding; junction stability; phosphorylation, internalization, degradation of VE-cadherin	130

Abbreviations: nm, nanometer; NP, nanopowder; NM, nanomaterials; A, anatase; R, rutile; and the number gives the mean diameter of NP. For example, TiO_2-A12 is an anatase TiO_2-NP, with a mean diameter of 12 nm. PEG, polyethyleneglycol; Tf, transferrin; Tc, titanocene;