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# Nanoparticles of Titanium and Zinc Oxides as Novel Agents in Tumor Treatment: a Review

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## Abstract

Cancer has become a global problem. On all continents, a great number of people are diagnosed with this disease. In spite of the progress in medical care, cancer still ends fatal for a great number of the ill, either as a result of a late diagnosis or due to inefficiency of therapies. The majority of the tumors are resistant to drugs. Thus, the search for new, more effective therapy methods continues. Recently, nanotechnology has been attributed with big expectations in respect of the cancer fight. That interdisciplinary field of science creates nanomaterials (NMs) and nanoparticles (NPs) that can be applied, e.g., in nanomedicine. NMs and NPs are perceived as very promising in cancer therapy since they can perform as drug carriers, as well as photo- or sonosensitizers (compounds that generate the formation of reactive oxygen species as a result of either electromagnetic radiation excitation with an adequate wavelength or ultrasound activation, respectively). Consequently, two new treatment modalities, the photodynamic therapy (PDT) and the sonodynamic therapy (SDT) have been created. The attachment of ligands or antibodies to NMs or to NPs improve their selective distribution into the targeted organ or cell; hence, the therapy effectiveness can be improved. An important advantage of the targeted tumor treatment is lowering the cyto- and genotoxicity of active substance towards healthy cells. Therefore, both PDT and SDT constitute a valuable alternative to chemo- or radiotherapy. The vital role in cancer eradication is attributed to two inorganic sensitizers in their nanosized scale: titanium dioxide and zinc oxide.

**Keywords:** Nanotechnology, Nanoparticles, Titanium dioxide, Zinc oxide, Cancer, Photodynamic therapy, Sonodynamic therapy

## Review

### Introduction

During the congress of the American Physical Society in Caltech, on 26th December 1959, an American physicist R. Feynman [1] held a presentation. He was the first to claim the matter can be altered at the level of atoms and particles. That statement, the main thesis of the speech entitled “*There’s plenty of room at the bottom,*” earned him the attribute of a forerunner of nanotechnology. In the lecture, R. Feynman suggested that in his day, the only limit to the development of nanotechnology resulted from the lack of sufficiently precise tools and techniques. For the first time, the term “nanotechnology” was applied by a Japanese scientist N. Taniguchi [2] and

meant the possibility of material engineering at the nanometers level. A book by K. Drexler [3] “*Nanosystems: molecular machinery, manufacturing and computation*” contributed to the popularization of the knowledge in this area. At present, nanotechnology is a rapidly developing multidisciplinary field that combines the latest achievements of many disciplines of science, including biology, chemistry, physics, informative technology, and mechanics. It designs nanomaterials (NMs) and nanoparticles (NPs) with at least one dimension ranging in the scale from 1 to 100 nm [4], confers on them the desired properties, and alters them accordingly. For comparison, the diameters of DNA, viruses, and lymphocytes are 2.5, 30–50, and about 7000 nm, respectively [5]. Nanotechnology, besides biotechnology and genetics, has recently become one of the most highlighted fields of science [6]. It gave rise to the creation of nanomedicine [7]. According to Fakruddin et al. [8], thanks to

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the achievements of nanotechnology, it will soon be possible to increasingly or even fully control the matter structure; hence, to overcome numerous obstacles, the medicine is nowadays struggling against. For example, by using quantum dots, nanotechnology might greatly assist in an early stage cancer diagnosis and therapy [9]. The regeneration and remodeling of damaged neuronal connections is going to be possible, thanks to dendrimers [10]. Carbon nanotubes can deliver drugs to cells in a more targeted manner [11]. Another example might be NM-based video capsule endoscopy providing high quality images of formerly inaccessible spots, e.g., small bowel [12].

The challenge the nanomedicine is now facing is to find the most effective way of the tumor cells eradication [13]. The main area this discipline is trying to explore is designing systems of targeted drug delivery and release into pathologically altered cells, increasing the drug treatment effectiveness, and limiting their side effects [14, 15]. There are several key attributes of NPs in their performance as drug carriers. One of them is their ability to become conjugated with ligands that are specific to cancer cells and that enable the targeted drug delivery [16–18]. NPs can be also conjugated with monoclonal antibodies, a process preventing them from immunological system response [19]. Moreover, thanks to the possibility of modifying the physicochemical properties of NPs, a smooth endocytosis into the cancer cells can occur [20]. NPs have also found their application in nanodiagnostics as biosensors for an early stage tumor markers detection [21] and in gene therapy as carriers delivering into cancer cells foreign nucleic acids that encode proteins killing the tumor cells [22].

The past decade brought about the discovery that many materials used in a variety of industries exhibit formerly undiscovered catalytic properties, after they have been powdered to NPs and subsequently exposed to ultraviolet (UV) radiation or ultrasound (US) wave. Among such materials, there are titanium dioxide ( $\text{TiO}_2$ ) and zinc oxide ( $\text{ZnO}$ ) [23–25].

### **Nanoparticles of Titanium and Zinc Oxides**

#### ***Size, Surface Area/Volume Ratio, and Surface Chemistry***

Nanoparticles of titanium dioxide ( $\text{TiO}_2$  NPs) and zinc oxide ( $\text{ZnO}$  NPs) are single elements with a diameter of  $1 < \phi \leq 100$  nm [4]. Their size is one of their key properties. The smaller the NP diameter, the bigger becomes the surface area/volume ratio. A high value of surface area/volume ratio allows a greater number of constituting atoms to be located around the surface of the NPs, a phenomenon making the NPs highly reactive and attributing them with new, unique physicochemical properties [26, 27]. Moreover, the smaller the NP diameter, the longer becomes the blood circulation time [28]. Metal

oxide nanoparticles whose diameter ranges between  $10 < \phi \leq 100$  nm are considered perfect in respect of their medical applications. The NPs with  $\phi \leq 10$  nm are able to deeper penetrate tumors and better accumulate in tumor cells, but they are also toxic to healthy cells. Bigger NPs, in the range of  $10 < \phi \leq 100$  nm, can only hardly be uptaken by healthy cells while they can easily penetrate tumor cells [29]. Nowadays, thanks to the nanoengineering, it is possible not only to precisely control the size of  $\text{TiO}_2$  and  $\text{ZnO}$  NPs but also to functionalize their surface. The NP functionalization provides for an easier transport within tissues, creates the NP ability to cross plasma membranes and allows them to be delivered to cancer cells in a targeted manner [30]. Thus, NPs of  $\text{TiO}_2$  and  $\text{ZnO}$  have become a promising tool in medicine [28–30].

#### ***Photo and Sonocatalytic Properties***

The electronic structure of  $\text{TiO}_2$  and  $\text{ZnO}$ , both belonging to the family of semiconducting metal oxides, determines their catalytic properties [31]. The semiconductors have a valence band (VB), which is filled with electrons, and an electron-free conduction band (CB). The energy difference ( $\Delta E$ ) between those bands is known as band gap, also referred to as energy gap. The band gap is an equivalent of the energy amount necessary to excite an electron from VB to CB. For nanosized titanium dioxide (nano- $\text{TiO}_2$ , anatase polymorph) and nanosized zinc oxide (nano- $\text{ZnO}$ ), the width of the energy gap is 3.20 and 3.37 eV, respectively [26, 32]. Both amounts approximate the value of the electromagnetic radiation photon energy with a wavelength of  $\lambda < 400$  nm. In biological trials, semiconducting metal oxides (e.g.,  $\text{TiO}_2$ ,  $\text{ZnO}$ ) are excited by the UV radiation (photoexcitation), mainly in the near ultraviolet range (UV-A,  $315 \leq \lambda < 400$  nm) [33–38]. Less common is the photoexcitation with a wavelength in the medium ultraviolet range (UV-B,  $280 \leq \lambda < 315$  nm) [33, 34, 36]. The use of far ultraviolet range (UV-C,  $100 < \lambda < 280$  nm) as an agent responsible for advanced oxidation processes may be hazardous to human health [37]. Another way to excite semiconductor NPs in aqueous solutions is the application of the US wave (sonoexcitation). The US wave ( $20 \text{ kHz} < f \leq 1 \text{ GHz}$ ), while propagating in the liquid, causes a twofold effect: a temporary punctual dilution (result of the loss of pressure), facilitating the formation of cavitation bubbles; as well as liquid's thickening (result of the rise of pressure) that facilitates the bubbles' collapse. This phenomenon is referred to as acoustic cavitation. It is accompanied by a number of secondary effects, among them sonoluminescence, an emission of sonoluminescent light (mainly UV light) during the implosion of the cavitation bubbles suspended in a liquid [39, 40].

The consequence of the semiconductor photo- or sonoexcitation is that electron ( $e^-$ ) transfers from VB to

CB, leaving behind a positive hole ( $h^+$ ). Therefore, a specific “electron-hole” pair ( $e^- + h^+$ ) is created [40–42]. This bound state, called exciton, lacks stability and exhibits redox properties. Charge carriers ( $e^-$ ,  $h^+$ ) can migrate to the surface of the catalyst (e.g.,  $TiO_2$  or  $ZnO$ ) NPs where they can be trapped by adsorbate molecules, initiating thereby the formation of reactive oxygen species (ROS). Excited negative electrons ( $e^-$ ) reduce molecular oxygen ( $O_2$ ), forming superoxide radical anion ( $\cdot O_2^-$ ). Generated positive holes ( $h^+$ ) oxidize water molecules ( $H_2O$ ) and hydroxide ions ( $OH^-$ ), producing hydroxyl radicals ( $\cdot OH$ ) and hydrogen peroxide molecules ( $H_2O_2$ ) [40–43] (Fig. 1).

The inverse process to the generation of charge carriers in semiconductor (e.g.,  $TiO_2$  or  $ZnO$ ) NPs is the decay of exciton. The recombination of the electron-hole pair ( $e^- + h^+$ ) is accompanied by the emission of a photon (radiative recombination). In this process, the excitation of ground state oxygen ( $O_2$ ) by photon occurs and singlet oxygen ( $^1O_2$ ) is formed [40, 43] (Fig. 1).

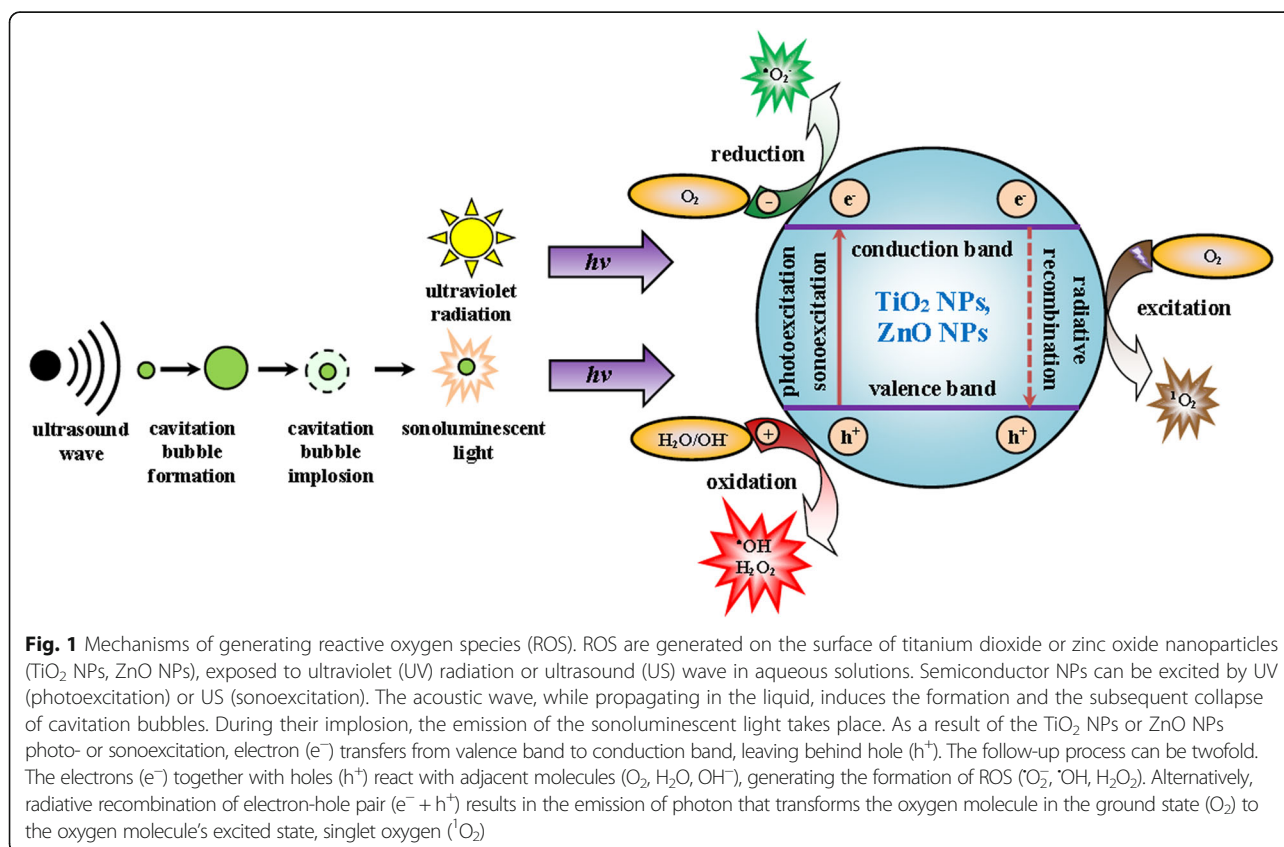
#### Oxidative Stress and its Anticarcinogenic Effects

The oxidative stress is an underlying factor for adverse biological effects caused by NPs of  $TiO_2$  and  $ZnO$ . In the cells, it is reflected by the increase of the level of ROS and oxidative products, as well as by the depletion

of cellular antioxidants [44]. ROS forming is an inherent element of the aerobic cellular metabolism. In their physiological concentrations, ROS perform a series of important functions. They mediate in the intracellular signal transduction [45], regulate protein phosphorylation [46], and control intracellular  $Ca^{2+}$  homeostasis [47]. Balance disruptions between the ROS formation and the efficiency of antioxidative systems lead to oxidative stress that poses a serious danger to the integrity of cells and their correct functioning [48].

Eukaryotic cells, similarly to prokaryotic cells, are equipped with mechanisms protecting them from ROS. The first defense line, preventing ROS from forming and from reactions with cell compounds, are proteins (e.g., lactoferrin) that sequester metal ions (e.g., Cu, Fe). Interruption of free radical chain reactions is the task of the second defense line that consists of a system of three enzymes: superoxide dismutase (SOD; EC 1.15.1.1), catalase (CAT; EC 1.11.1.6), and glutathione peroxidase (GPX; EC 1.11.1.9), supported by low molecular weight antioxidants, such as glutathione (GSH), thioredoxin (Trx), or coenzyme  $Q_{10}$  ( $CoQ_{10}$ ). The third defense line present repair enzymes responsible for nucleic acids damage overhaul, e.g., DNA ligase (EC 6.5.1.1) [48].

ROS can inflict cell injury as an effect of reaction with lipids in cellular membranes [49], nucleotides in DNA



[50], or sulfhydryl groups in proteins [51]. Depending on the intensity of the oxidative stress, the cell can undergo carcinogenesis or enter one of the cell death pathways [52]. Antunes and Cadenas [53] proved that as the concentration of certain ROS (e.g.,  $H_2O_2$ ) increases, the cell's viability decreases. In physiological conditions, the concentration of  $H_2O_2$  is low and ranges within 5–50 nM. The gradual increase of its quantity in a cell up to the level of 0.7  $\mu M$  triggers mechanisms that result in a cell proliferation and tumor formation. In case the  $H_2O_2$  concentration reaches the value of 1–3  $\mu M$ , the apoptosis or autophagy (programmed cell death pathways) induction occurs. The concentration in excess of the level of 3  $\mu M$  becomes toxic for a cell hence leads to its necrosis (unprogrammed cell death pathway). Therefore, the phenomenon of the oxidative stress in tumor cells has recently been used to design new tumor therapies [54, 55].

Increasingly frequent are the opinions that in near future, the NPs of semiconductor metal oxides (e.g.,  $TiO_2$  and ZnO) are going to play the crucial role in medicine as photo- or sonosensitizers applied in ROS-based cancer therapy [56, 57], a finding that naturally corroborates the necessity to study their cyto- and genotoxicity.

#### **Toxicity of Titanium Dioxide and Zinc Oxide Nanoparticles**

Despite the assumption that the micro-sized titanium dioxide (micro- $TiO_2$ ) is harmless to people and animals [58, 59], the issue of  $TiO_2$  toxicity has been a research subject in many scientific centers. Recent years have witnessed a dynamic development of nanotechnology. Along with it, there has been an increased concern, though that this compound might be toxic in its NPs form. There are studies proving that NPs ( $1 < \phi \leq 100$  nm) of any compound are more harmful to people and animals than microparticles (MPs) ( $0.1 < \phi \leq 100$   $\mu m$ ) of the same compound [60, 61].

#### **Studies In Vivo**

Wang and Fan [62] conducted studies showing that the level of the rats' pulmonary cell impairment, inflicted by the intratracheally instilled  $TiO_2$ , clearly depended upon the diameter of its particles. Kobayashi et al. [63] reported the pulmonary toxicity of  $TiO_2$  NPs in rats, evaluated on the basis of the short-term post-installation lung injury (up to 7 days after the installation), increased proportionally to the fragmentation of the  $TiO_2$  particles. Sycheva et al. [64] pointed out the genotoxicity of  $TiO_2$  NPs ( $\phi = 30$  nm), as measured by the number of DNA double-strand breaks and the number of micronuclei, was significantly higher in brain, liver, or bone marrow cells in mice, compared to the genotoxicity of  $TiO_2$  MPs ( $\phi = 160$  nm). Danio rerio, when exposed to  $TiO_2$

(50 mg  $dm^{-3}$ ) in their NPs form, exhibits more serious oxidative injuries of liver and gill cells, in comparison to the exposition to the MPs form of those compounds [65]. The studies by Ma-Hock et al. [66] showed the rats' exposition to the aerosol of  $TiO_2$  NPs (50 mg  $m^{-3}$ ) caused lung inflammation, accompanied by the increase of total neutrophil count in bronchoalveolar lavage (BAL) fluid. Bermudez et al. [67] examined pulmonary responses in rats, mice, and hamsters after their exposition to the aerosol of  $TiO_2$  NPs (10 mg  $m^{-3}$ ). They concluded there was a clear increase of cytotoxicity markers, i.e., an increase in the total macrophage and neutrophil count in BAL fluid in rats and mice, while in hamsters, there were no distinct changes in the cellular response. Ze et al. [68] proved the  $TiO_2$  NPs, administered to mice through the nose for 90 days, moved up and built up in the brain, leading to the oxidative stress, excessive glial cells proliferation, and tissue necrosis, as well as inflicting hippocampal cells apoptosis. Lindberg et al. [69] reported that the intraperitoneal administration of  $TiO_2$  NPs to mice resulted in an increase of DNA damages in leukocytes and in higher micronuclei count in peripheral blood polychromatic erythrocytes. The intratracheal instillation of  $TiO_2$  NPs showed the same effects in BAL cells. The cyto- and genotoxicity of  $TiO_2$  NPs were confirmed by many other authors [70–73]. Ferin and Oberdörster [74] showed the dust of  $TiO_2$  NPs, introduced into the trachea of a rat in the amount of 5 mg per animal, caused respiratory tract cancer within 4 months. Therefore,  $TiO_2$  NPs in the dust form, contrary to the  $TiO_2$  MPs in the same form, were classified in the 2B group by the International Agency for Research on Cancer (IARC) [75]. The 2B group assembles compounds that might be carcinogenic for humans.

#### **Studies In Vitro**

One of the main differences between  $TiO_2$  MPs and  $TiO_2$  NPs is a much bigger active surface area of  $TiO_2$  NPs, a feature resulting in a higher absorption rate of UV radiation and a greater photocatalytic activity [41]. Gurr et al. [76] prove there is a strong correlation between the  $TiO_2$  NPs genotoxicity and their diameter.  $TiO_2$  NPs ( $\phi < 50$  nm) caused the formation of 8-hydroxy-2-deoxyguanosine (8-OHdG) adducts and the increase of the micronuclei number in human bronchial epithelial cells, while  $TiO_2$  MPs ( $\phi > 200$  nm) in the same concentration were practically harmless. Kongseng et al. [77] report  $TiO_2$  NPs ( $\phi < 30$  nm) were responsible for the rise of the pro-inflammatory cytokine (IL-1 $\beta$  and TNF $\alpha$ ) level in human peripheral blood mononuclear cells (PBMCs) in serum-free medium. The in vitro studies of Long et al. [78, 79], conducted on mouse brain microglia cells (BV2 line), proved that  $TiO_2$  particles

with  $10 \leq \phi \leq 20$  nm stimulated the generation of ROS and, as a consequence, induced damages of neurons, whereas UV radiation was not a precondition for generating ROS. There are, however, numerous contrary opinions. Fujita et al. [80], drawing on DNA microarray analysis of human keratinocytes (HaCaT line), proved that the natural (with no UV irradiation) toxicity of TiO<sub>2</sub> NPs was very low. Similar conclusions were presented by Verves and Jha [81] who examined in vitro the toxicity of TiO<sub>2</sub> NPs (50 mg mL<sup>-1</sup>) to rainbow trout (*Oncorhynchus mykiss*) gonadal tissue cells (RTG-2 line). They reported cyto- and genotoxic effects of TiO<sub>2</sub> NPs in irradiated cells, only. Saquib et al. [82], while examining the toxicity of TiO<sub>2</sub> NPs (10 µg mL<sup>-1</sup>) to UV-A irradiated human amniotic epithelial cells (WISH line), noted a decrease in the CAT activity, lowering the GSH level, as well as the arrest of the cellular cycle in the G<sub>2</sub>/M phase. In similar studies on human epidermoid carcinoma cells (A431 line), Shukla et al. [83] observed an increase in lipid hydroperoxide (LPO) concentration, numerous oxidative DNA damages, and the formation of many micronuclei. Vamanu et al. [84] exposed human leukemic monocyte lymphoma cells (U937 line) in vitro to 0.1% colloidal nano-TiO<sub>2</sub> for 120 min and, subsequently, to UV-A irradiation. After 30 min of UV-A irradiation, significant plasma membrane damages and DNA fragmentation were noticed. Other studies show that TiO<sub>2</sub> powdered to NPs exhibited ROS-dependent cyto- and genotoxicity towards human HaCaT cells [85], protozoan (*Cryptosporidium parvum*) oocysts [86], as well as towards cysts of *Giardia lamblia* [87] and *Acanthamoeba castellanii* [88], only if the parasites were exposed to UV radiation. The cytotoxicity was determined on the basis of the lysosomal membrane stability, using neutral red retention time (NRRT) assay whereas the genotoxicity was measured by the number of DNA double-strand breaks, using the single-cell gel electrophoresis (SCGE).

ZnO toxicity is also dependent on the scale of its fragmentation [65]. Microsized zinc oxide (micro-ZnO), similarly to micro-TiO<sub>2</sub>, is practically harmless to people and animals [75], contrary to nano-ZnO. Wang et al. [89] studied the toxicity of ZnO NPs to human HaCaT cells and discovered the viability of those cells was clearly reduced, the integrity of their membranes was disrupted, and numerous oxidative DNA damages occurred. Those effects significantly increased when the keratinocytes were exposed to the UV radiation. Similar cyto- and genotoxicity effects of ZnO NPs were observed in human monocytes (THP-1 line) [90, 91] and human lung adenocarcinoma epithelial cells (A549 line) [92], as well as in fish embryos, e.g., *Danio rerio* [93].

Cyto- and genotoxicity of TiO<sub>2</sub> NPs, as well as of ZnO NPs have been proved in vitro also with many other cells, both healthy, e.g., human dermal fibroblasts

[94, 95], human periodontal ligament fibroblasts [96], human alveolar macrophages [97], human gastric epithelial cells [98]; and malignant, e.g., human colonic mucinous adenocarcinoma cells (Ls-174-t line) [99], human colon adenocarcinoma cells (LoVo line) [100, 101], human cervical carcinoma cells (HeLa line) [102–105], human breast adenocarcinoma cells (MDA-MB-468 line) [106], human hepatocellular carcinoma cells (Bel 7402 line) [107], human brain glioblastoma cells (T98G line) [108, 109], or human bladder carcinoma cells (T-24 line) [110].

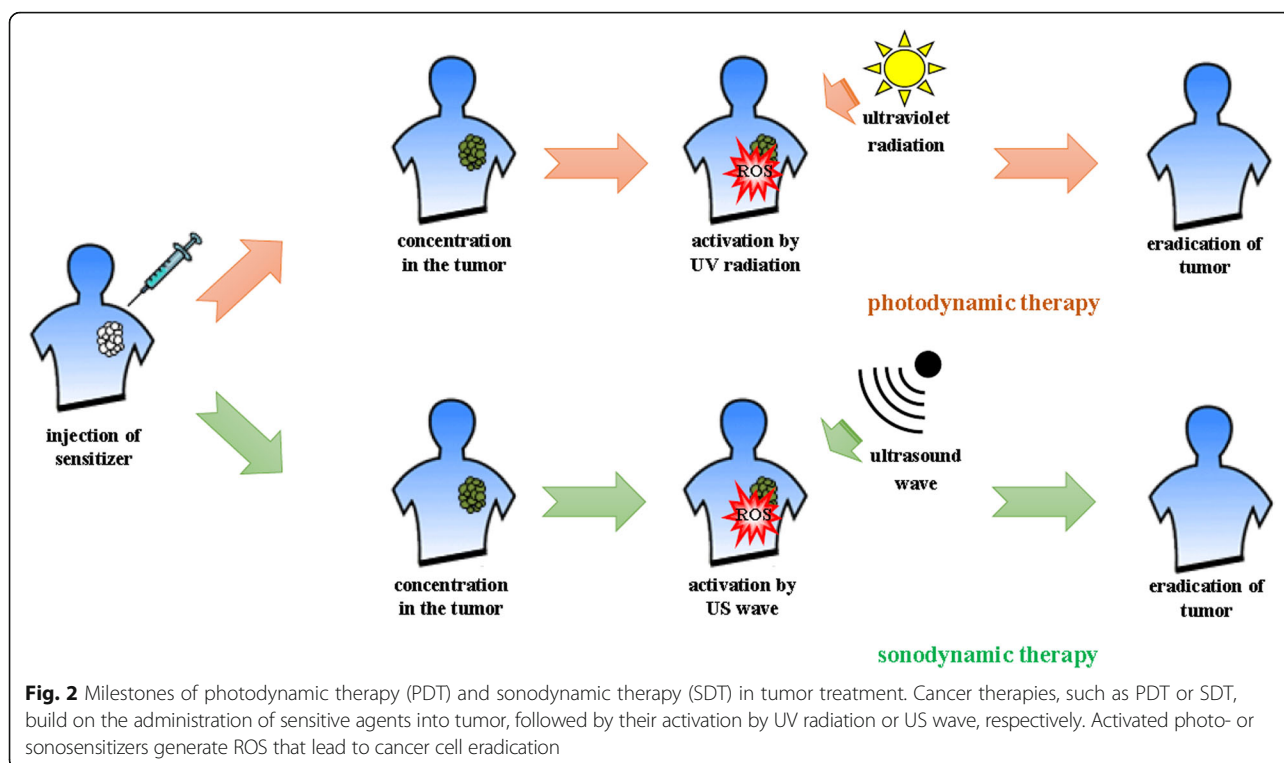
#### Future of Cancer Therapy in View of the Application of Titanium Dioxide and Zinc Oxide Nanoparticles

In recent years, there has been an increase in cancer disease prevalence, an issue inducing an acute need to invent new, effective, and safe therapy methods. In the past decade, nanotechnology and nanomedicine gained quite an importance in anticancer therapy. Those two fields of science joined forces not only to seek substances that would effectively eradicate cancer cells but also to find ways of delivering chemotherapeutics to tumor cells, in a targeted manner [111, 112]. The introduction of nanosized photo- and sonosensitizers to the medicine gave rise to develop new therapeutic strategies, photodynamic therapy (PDT) [113] and sonodynamic therapy (SDT) [114], respectively. Designing of new ways of NM-based targeted drug delivery into cancer cells (targeted therapy) lowered side effects inflicted by many marketed drugs, such as doxorubicin and daunorubicin [115–117]. Among the scientists, great hopes to fight cancer more effectively are attributed to the application of NPs of metal oxides (e.g., TiO<sub>2</sub>, ZnO) in PDT [113, 118, 119], a treatment strategy presenting an alternative to chemo- and radiotherapy.

#### Photodynamic Therapy

PDT is a treatment in which the administration of photosensitizer to the tumor tissue is followed by the excitation of a photosensitizer by electromagnetic radiation with a specific wavelength, e.g., UV-A or UV-B. Consequently, on the surface of NPs of photoexcited metal oxides, ROS are formed which then damage cancer cells [120] (Fig. 2).

Photosensitizers, such as TiO<sub>2</sub> or ZnO NPs, can be delivered to the tumor cells as the result of passive or active processes [121]. The passive process takes advantage of the enhanced permeability and retention (EPR) effect. Leaky vasculature in the cancer tissue allows the NPs to be freely uptaken into the tumor cells, a phenomenon leading to the cell death. The uptake takes place also in the inflamed tissue; hence, the passive process does not provide for the targeted NPs delivery to the tumor cells [122]. In the active process, the functionalization of NPs occurs; therefore, the NPs can be

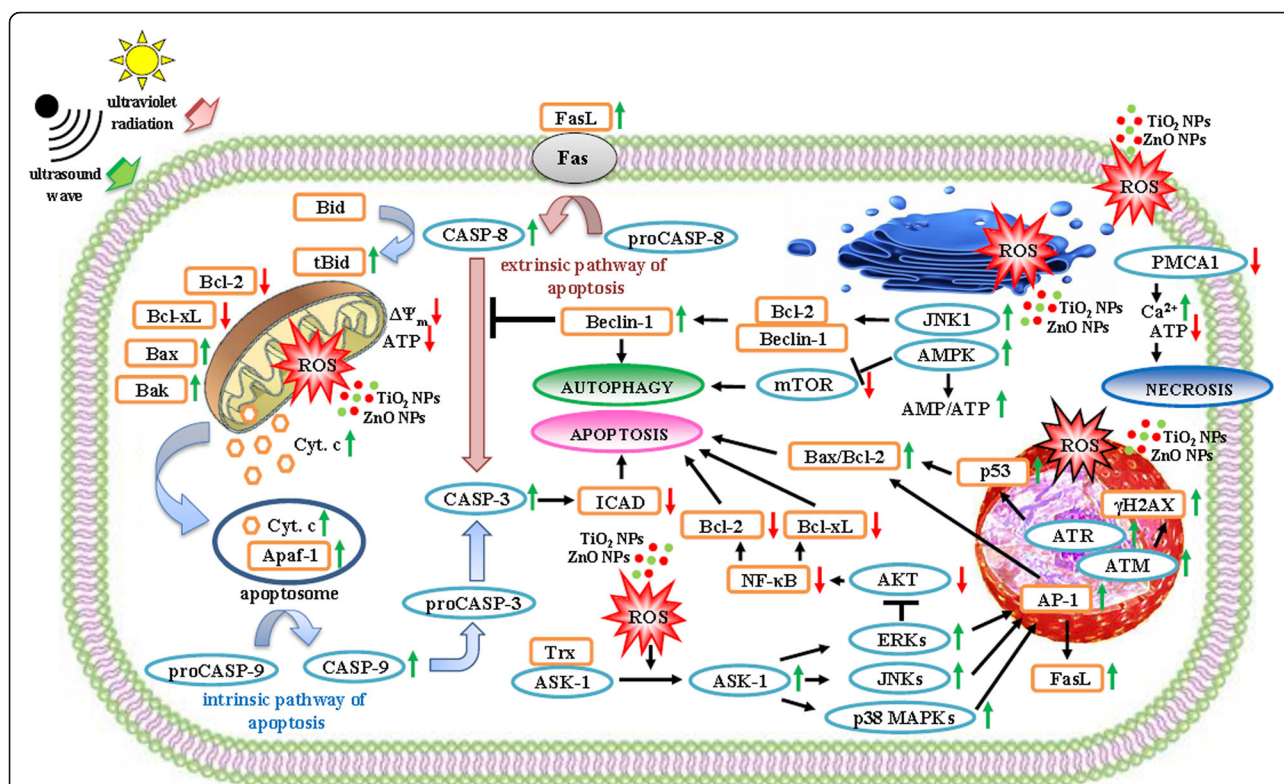


delivered to tumor cells in a targeted manner. The functionalization of NPs means their conjugation with specific antibodies or ligands. PDT clearly lowers the cancer therapy side effects, compared to chemo- or radiotherapy [123]. The way the cellular uptake of nanoparticles takes place, their subcellular position and the ability to cause toxic effects depend mainly upon the NPs diameter, their surface area, surface chemistry, and charge [124]. There are two major ways of the NPs uptake into the cell: active uptake by endocytosis and passive uptake by diffusion. Due to their strong hydrophilic properties, both  $\text{TiO}_2$  and  $\text{ZnO}$  NPs are uptaken into the cells by phagocytosis [125].

It is assumed that  $\text{TiO}_2$  NPs, if exposed to the UV radiation, induce, depending on their subcellular position, one of the cell death pathways: apoptosis [105, 126], autophagy [127, 128], or necrosis [129, 130], whereas the mechanism of those processes is still insufficiently examined (Fig. 3).

Zhang et al. [131], having examined UV-excited  $\text{TiO}_2$  NPs toxicity towards human hepatocarcinoma cells (SMC-7721 line), reported an increase and a decrease of protein levels that are, correspondingly, pro- (e.g., Bax, Bak, tBid) and anti-apoptotic (e.g., Bcl-2, Bcl-xL) molecules. Zhao et al. [132] point out there is an overexpression of the tBid in the  $\text{TiO}_2$  NPs-treated mouse epidermal cells (JB6 line), this protein playing a crucial role in the involvement of mitochondria in oxidative

stress-dependent cell death. PDT-induced mitochondria damages result in a selective Bcl-2 degradation, with no effect on the Bax. Therefore, the Bax/Bcl-2 ratio is increased, and the intrinsic pathway of apoptosis is promoted [133–135]. Those effects were noticed in PDT-treated human breast epithelial cells (MCF10A line) [136], human brain glioblastoma cells (U87MG line) [137] or human gastric adenocarcinoma cells (MGC803 line) [138]. Nearly identical changes in levels of proteins regulating apoptosis were observed by Ahamed et al. [139] and by Wahab et al. [140] whereby the purpose of their experiments was to examine the  $\text{ZnO}$  NPs toxicity towards human A549 cells, as well as human breast adenocarcinoma cells (MCF-7 line) and human hepatocellular liver carcinoma cells (HepG2 line). The level of Bax and Bcl-2 proteins in  $\text{ZnO}$  NPs-treated ( $50 \mu\text{g mL}^{-1}$ , 24 h) human HepG2 cells was twofold higher and threefold lower, respectively, compared to control cells [140]. The principle function of Bax is the formation, with the assistance of Bak, of pores in the outer mitochondrial membrane that enhance its permeability. Effectively, the release of cytochrome c (Cyt. c) from the mitochondrial intermembrane space into the cytosol follows. Cyt. c, along with the apoptotic protease-activating factor 1 (Apaf-1), contributes to the formation of the structure called apoptosome. This structure activates the initiator caspase-9 (CASP-9; EC 3.4.22.62) that subsequently activates the



**Fig. 3** Molecular mechanisms of reactive oxygen species (ROS)-mediated cell death pathways. Activated by ultraviolet (UV) radiation or ultrasound (US) wave, nanoparticles of titanium dioxide (TiO<sub>2</sub> NPs) and zinc oxide (ZnO NPs) induce ROS-mediated cell apoptosis, autophagy or necrosis. The increase of ROS level in cytoplasm results in thioredoxin (Trx) oxidation and apoptosis signal-regulating kinase 1 (ASK1) activation. ASK1 phosphorylates mitogen-activated protein kinases (ERKs, JNKs, p38 MAPKs) that control the AP-1-mediated synthesis of pro- (Bak, Bax) and anti-apoptotic (Bcl-2, Bcl-xL) proteins, as well as death ligands (FasL), thus promoting the cell apoptosis. Likewise, ERKs promote the cell apoptosis through attenuation of activity of AKT kinase that controls the NF- $\kappa$ B-mediated synthesis of Bcl-2 and Bcl-xL proteins. The oxidative damages of mitochondrion result in dissipation of mitochondrial membrane potential ( $\Delta\Psi_m$ ), decrease of ATP level and release of cytochrome c (Cyt. c) to the cytosol. Cyt. c, binding with apoptotic protease activating factor 1 (Apaf-1), induces the caspase-dependent apoptosis. The increase of ROS level in nucleus results in the activation of ataxia telangiectasia mutated (ATM), as well as ataxia telangiectasia and Rad 3-related (ATR) kinases that induce the p53-mediated apoptosis and account for the increase of DNA double-strand breaks marker ( $\gamma$ H2AX) level. The ROS-induced endoplasmic reticulum stress results in the JNK1-mediated increase of pro-autophagic protein (Beclin-1) level and the AMPK-mediated inhibition of autophagic inhibitor, mammalian target of rapamycin (mTOR) kinase. Both processes lead to the cell autophagy. The oxidative injuries of plasma membrane result in the downregulation of plasma membrane calcium ATPase 1 (PMCA1), in the decrease of ATP level and the increase of Ca<sup>2+</sup> level. As a consequence, the cell necrosis follows

executioner caspase-3 (CASP-3; EC 3.4.22.56). Bcl-2 is an inhibitor of apoptosis. It associates with the outer mitochondrial membrane, keeping therefore its integrity [141, 142] (Fig. 3).

According to many authors [139, 140, 143, 144], the key role in the cell death induced by the UV-excited NPs of TiO<sub>2</sub> or ZnO is played by ROS. They are strong oxidants but also important agents modulating ROS-sensitive signaling pathways that lead to the cell death. Biola-Clier et al. [145] report the ROS-mediated downregulation of DNA repair processes such as base excision repair and nucleotide excision repair. Toyooka et al. [146], when examining toxicity of TiO<sub>2</sub> NPs (15  $\mu$ g mL<sup>-1</sup>) to human A549 cells, concluded there was an increased H2AX histone phosphorylation that followed the oxidative stress-induced DNA double-strand breaks. Similar results obtained Premanathan

et al. [147], having examined human HL-60 cells exposed to ZnO NPs (10  $\mu$ g mL<sup>-1</sup>). Phosphorylated H2AX ( $\gamma$ H2AX) histone is considered to be a marker of DNA damages [148]. Its phosphorylation is caused by ataxia telangiectasia mutated kinase (ATM; EC 2.7.11.1), as well as by ataxia telangiectasia and Rad 3-related kinase (ATR; EC 2.7.11.1), enzymes participating in the DNA damage response (DDR) pathway. The key effector protein on this pathway is p53. The importance of the ROS-induced activation of p53-mediated DNA damage check point signals in apoptosis of TiO<sub>2</sub> NP-treated human peripheral blood lymphocytes (PBL) was stressed by Kang et al. [143]. Responding to the big scale of the DNA damages, p53 induces the pro-apoptotic protein gene expression, followed by the cell death [143] (Fig. 3).

Yoo et al. [144] studied the ROS mediation in the induction of both intrinsic and extrinsic apoptotic

pathways since ROS stimulate the increase of pro-apoptotic protein (Bax) and death ligand (FasL) levels, respectively. ROS induce apoptosis in tumor cells also by modification of the activity of mitogen-activated protein kinases (MAPKs; EC 2.7.11.24) [149] and of the nuclear factor kappa B (NF- $\kappa$ B) [150]. Cheng et al. [151] report that PDT-generated ROS modify the ability of thioredoxin (Trx), the intracellular sensor of oxidative stress, to inhibit the apoptosis signal-regulating kinase 1 (ASK1; EC 2.7.11.25). ASK1 forms an inactive structure with reduced Trx. In the presence of ROS, the oxidation of Trx takes place and the structure degrades. The free form of ASK1 phosphorylates MAPKs such as c-Jun N-terminal protein kinase isoform 1 and 2 (JNK1/2), p38 mitogen-activated protein kinases (p38 MAPKs), and extracellular signal-regulated kinase isoform 1 and 2 (ERK1/2) [152]. Phosphorylated MAPKs translocate into the nucleus, where they induce the formation of the activator protein 1 (AP-1), a transcription factor regulating the expression of the AP-1-dependant pro- and anti-apoptotic genes [153]. JNK1/2 and p38 MAPKs lead to AP-1-mediated inactivation of anti-apoptotic proteins (Bcl-2 and Bcl-xL), the result of which is the formation of apoptosome and the activation of caspases cascade, both subsequently leading to the proteolysis of inhibitor of caspase-activated DNase (ICAD) and then, as a consequence, to the DNA fragmentation [154, 155]. JNK1/2 and p38 MAPKs promote apoptosis in case the ERK1/2 are inhibited or remain inactive [156, 157]. This conclusion was confirmed by the studies on the cytotoxicity of ZnO NPs to human HepG2 cells performed by Yuan et al. [158]. Nevertheless, in oxidative stress conditions, ERK1/2 might adopt a pro-apoptotic role since they attenuate the activity of protein kinase B (PKB, EC 2.7.11.1), called also AKT1 [159]. This phenomenon was observed in renal proximal tubule epithelial cells (LLC-PK1 line) [160]. AKT1 activates NF- $\kappa$ B through I $\kappa$ B kinase (IKK; EC 2.7.11.10) phosphorylation [161]. Coupienne et al. [162] suggest that ROS induce the AKT-mediated inactivation of NF- $\kappa$ B, an inhibitor of apoptosis. The NF- $\kappa$ B activation occurs in the cytoplasm and follows the detachment of the  $\kappa$ B inhibitor (I $\kappa$ B). The active form of NF- $\kappa$ B translocates into the nucleus, where it initiates expression of genes that encode anti-apoptotic proteins (Fig. 3). The studies by Granville et al. [163] revealed PDT-treated human promyelocytic leukemia cells (HL-60 line) showed no detectable I $\kappa$ B level in the cytoplasm. It might constitute an explanation of typical morphological symptoms of the apoptotic cell death, as noticed using the confocal laser scanning microscope (CLSM), such as nucleus fragmentation, cytoplasm shrinkage, apoptotic bodies formation, or lack of inflammation reaction.

PDT-induced damages of the endoplasmic reticulum habitually result in the cell death by autophagy [164] (Fig. 3). In the oxidative stress, the activation of JNK1 that phosphorylates Bcl-2 occurs. Consequently, the dissociation of Beclin 1/Bcl-2 complex, hence the activation of Beclin 1, a pro-autophagic protein, takes place [165–167]. Such effects were noticed in PDT-treated human prostate adenocarcinoma cells (DU-145 and PC3 lines) [168]. Beclin 1 inhibits apoptosis by the inactivation of caspase-8 (CASP-8; EC 3.4.22.61), an enzyme transforming Bid to tBid, a pro-apoptotic protein. Beclin 1 also contributes, along with the phosphatidylinositol 3-kinase class III (PI3K3C; EC 2.7.1.137), towards the formation of the autophagosome that eliminates cytoplasmic “waste” material [166]. Autophagosome fuses with lysosome, leading therefore to the formation of autophagolysosome, in which lysosomal hydrolases complete the process of the definite destruction of its content [169–171]. Among the morphological symptoms of the autophagic cell death, there are increased number of autophagic vesicles, the degradation of Golgi apparatus, and endoplasmic reticulum, as well as no inflammation reaction [164]. Those symptoms were observed by Yu et al. [128] in TiO<sub>2</sub> NP-treated human bronchial epithelial cells (16HBE140-line). High level of ROS in a cell results in the increase of the AMP/ATP ratio, an effect stimulating the AMP-activated protein kinase (AMPK; EC, 2.7.11.31) [172, 173]. AMPK may efficiently inhibit the mammalian target of rapamycin kinase (mTOR; EC. 2.7.11.1). mTOR is a key autophagy inhibitor and is frequently overexpressed in miscellaneous tumor cells [174, 175]. Its PDT-induced inhibition, leading up to the autophagy, was observed in human breast adenocarcinoma cells (MDA-MB-231 line) [176].

High level of the PDT-generated ROS, inflicting pro-apoptotic (Bax, Bak) and pro-autophagic (Beclin 1) protein damages, as well as plasma membrane disruption, results in a cell death by necrosis [177, 178] (Fig. 3). Cell membrane oxidative damages cause the increase of the cytosolic Ca<sup>2+</sup> level (>1  $\mu$ M) that activates Ca<sup>2+</sup>-dependent proteases. Other effects are dissipation of mitochondrial membrane potential ( $\Delta\psi_m$ ) and a dramatic decrease of the intracellular ATP level [178]. Guo et al. [179, 180] exposed rat retinal ganglion cells (RGC-5 line) to ZnO NPs and concluded there was a remarkable increase in Ca<sup>2+</sup> level in cytosol that resulted from the downregulation of plasma membrane calcium ATPase isoform 1 (PMCA1; EC 3.6.3.8) activity. Disrupted homeostasis of Ca<sup>2+</sup> was also observed in human lens epithelial cells (HLE-B3 line) incubated with ZnO NPs and, subsequently, exposed to UV-B radiation [181]. Typical morphological symptoms of the necrotic cell death encompass: cell swelling, destruction of nucleus and of other cell organelles, plasma membrane disruption—all of them



resulting in a cell disintegration, followed by the release of the cell content into the intercellular space hence inflammation reaction [182]. Xu et al. [108] described the necrosis of human lung adenocarcinoma cells (APC-A1 line), induced by photoexcited TiO<sub>2</sub> NPs.

PDT-treatment of tumor cells, using the metal oxide nanoparticles, results in the cell death, presenting the consequence of irreversible DNA damage [146], plasma membrane disintegration [183], intracellular Ca<sup>2+</sup> homeostasis disruption [179, 180], strong reduction or complete exhaustion of the redox compound pool, essential in ATP synthesis [184]. The main factor of reported damages is PDT-induced oxidative stress [184–187].

Nanocomposites, e.g., gold-doped TiO<sub>2</sub> (Au/TiO<sub>2</sub>) [101], silver-doped TiO<sub>2</sub> (Ag/TiO<sub>2</sub>) [102], platinum-doped TiO<sub>2</sub> (Pt/TiO<sub>2</sub>) [188], or silver-doped ZnO (Ag/ZnO) [189] are compounds increasingly often applied in anticancer therapy. The conjugation of NPs of a precious metal with TiO<sub>2</sub> NPs increases the catalytic activity of this oxide, a phenomenon confirmed in the studies of Abdulla-al-Mamun et al. [102]. They reported that Ag/TiO<sub>2</sub> nanocomposite showed an 80% higher effectiveness in eradication of human HeLa cells than TiO<sub>2</sub> NPs. In other studies, TiO<sub>2</sub> NPs were conjugated with folic acid (FA) molecules [190]. Such conjugation enhanced the ability of human HeLa cells to uptake FA/TiO<sub>2</sub> nanocomposite by FA receptors in plasma membrane and, therefore, to leverage the efficiency of cancer cells eradication. Feng et al. [191] confirmed high biocompatibility of FA/TiO<sub>2</sub> nanocomposite and its incorporation into human nasopharyngeal carcinoma (KB) cells. Rozhkova et al. [109] designed TiO<sub>2</sub>-based nanocomposites that target and eradicate brain carcinoma cells in vivo with no harm to adjacent healthy cells. IL13 $\alpha$ 2R antibodies were conjugated with TiO<sub>2</sub> NPs through 3,4-dihydroxyphenylacetic acid (DOPAC). The antibodies recognize cancer cells, bind to them and are eventually uptaken by means of phagocytosis. In the process of tumor cells exposition to UV-A radiation, TiO<sub>2</sub>-based nanoconstructs generate the formation of ROS that subsequently damage the cancer cells and induce their apoptosis. The selection of suitable antibodies can help design nanocomposites able to precisely target and eradicate many types of cancer cells [82, 83]. Xu et al. [100] advocate a simultaneous application of conjugates of TiO<sub>2</sub> NPs with monoclonal antibodies, along with reversible plasma membrane injury by means of electric field, so called electroporation. As the authors suggest, such combination may increase the accuracy of cancer cell recognition by TiO<sub>2</sub>-based nanocomposites and, in consequence, enhance the TiO<sub>2</sub>-based nanoconstructs penetration into cytosol. Using that method, it was possible to eradicate in vitro all human LoVo cells that were subject to experiment, within merely 90 min.

One of the main disadvantages of PDT is the limited penetration depth of the tissues by the UV radiation. Therefore, this method is suitable to eradicate tumor cells located in superficial depths [192]. This shortcoming might be rectified, and deeper located tumor cells can still be eradicated using PDT, if NPs of TiO<sub>2</sub> and ZnO are conjugated with upconverting nanoparticles (UCNPs) [193]. Ytterbium and erbium doped sodium yttrium fluoride (NaYF<sub>4</sub>:Yb,Er) is frequently applied as transducer [194]. UCNPs absorb near infrared (NIR, 700  $\leq$   $\lambda$  < 850 nm) radiation and emit UV ( $\lambda$  < 400 nm) radiation. NIR radiation, compared to UV radiation, penetrates tissues in bigger depths but is also less harmful to the organism. Therefore, UCNP-based PDT gains enhanced effectiveness in the eradication of deeper depths tumor cells [195]. This finding has been confirmed both in vitro, in human cancer cells (HeLa and MCF-7 lines) and in vivo, in female Balb/c nude mice [196, 197].

Thevenot et al. [198] withdrew from TiO<sub>2</sub> NPs excitation by UV radiation in favor of the chemical functionalization of their surfaces by binding to them –OH, –NH<sub>2</sub>, or –COOH groups. Then, they examined TiO<sub>2</sub> NPs, as modified on their surface, in respect of their influence on the viability of a number of cancer cell lines (e.g., B16F10 mouse melanoma cell line, 3T3 mouse embryonic fibroblast cell line, or PC-3M human prostate carcinoma cell line) in vitro. According to the authors, the viability of the examined cancer cell lines was dependent upon both the concentration of TiO<sub>2</sub> NPs in the examined cells and the type of functional group altering the surface properties of TiO<sub>2</sub> NPs. The highest cyto- and genotoxicity exhibited NPs functionalized by –OH groups. The design of surface properties of NMs and NPs presents a valuable tool in the targeted cancer therapy.

#### **Sonodynamic Therapy**

Limited possibilities to apply PDT in the cancer therapy gave rise to seek different cancer treatment modalities. An example of an alternative tumor eradication method is SDT. This therapy is based on a synergistic effect on a cancer cell by sonosensitizer and US [199]. US can activate particles of compounds called sonosensitizers. In comparison with UV or NIR radiation, US penetrates tissues much more effectively as it reaches deeper depths tumor cells. The introduction of sonosensitizers into cancer cells and their subsequent exposure to US in order to generate ROS is the underlying factor of SDT [199, 200] (Fig. 2).

Harada et al. [114] showed in their studies in vitro that there was a remarkable reduction (in excess of 50%) in viability of human melanoma cells (C32 line) upon exposure to the TiO<sub>2</sub> NP-based SDT (1 MHz, 30 s). Moosavi Nejda et al. [200] examined in vitro the influence

of the US-excited TiO<sub>2</sub> NPs (100 µg mL<sup>-1</sup>) on the viability of human oral squamous carcinoma cells (HSC-2 line). The authors proved the increased intensity of US (20, 32, 55, 73 W cm<sup>-2</sup>) and the prolonged sonication period (0.1, 1.0, 3.0 s) enhanced the damage ratio in the human HSC-2 cells, finally leading to their necrosis at the conditions of 73 W cm<sup>-2</sup> and 3 s. Yamaguchi et al. [183] believe that TiO<sub>2</sub> NP-based cancer therapies will soon become a valuable tool in fighting malignant gliomas. The authors compared PDT to SDT in respect of their effectiveness in eradication of human glioblastoma cells (U251 line). With PDT, the toxicity effect of TiO<sub>2</sub> NPs (20 µg mL<sup>-1</sup>) was noticeable after 2-h exposure of single cancer cell layer to UV-A radiation (5.0 mW cm<sup>-2</sup>). Moreover, the effect was fairly restricted by free radical scavengers. Cytotoxicity of TiO<sub>2</sub> NPs (20 µg mL<sup>-1</sup>) in case of SDT was proportional to the sonication period, and it was not dependent on antioxidants concentration, an observation indicating there must be a different mechanism of intracellular structure damages. US, propagating in cell, induce the formation of cavitation bubbles which free energy as they implode. It is assumed that acoustic cavitation can not only lead to sonosensitizer excitation, the result of which is <sup>1</sup>O<sub>2</sub> formation, but it can also cause—with no ROS involved—a disruption in plasma membrane integrity. Saito et al. [201] report that the necrotic death of human corneal endothelial cells after the US exposure can take place rapidly and could be attributed to mechanically induced damage to the plasma membrane. Thanks to fluorescent dyes, a greater amount of plasma membrane damages resulting from the TiO<sub>2</sub> NP-based SDT could be noticed than in the TiO<sub>2</sub> NP-based PDT.

In SDT, similarly to PDT, TiO<sub>2</sub> NPs are also conjugated with precious metals or antibodies. The subject of studies conducted by Ogino et al. [202], as well as Ninomiya et al. [203, 204], was the examination of TiO<sub>2</sub> NP toxicity to human HepG2 cells. To increase the TiO<sub>2</sub> NPs uptake rate by cancer cells, pre-S1/S2, a model antibody recognizing hepatocytes, was conjugated with the TiO<sub>2</sub> NPs. The ability of such conjugates to recognize cancer cells was examined using the surface plasmon resonance and immunofluorescence techniques. Apoptosis of some cells of the human HepG2 cells in vitro was firstly observed after 6 h from the termination of the TiO<sub>2</sub> NP sonication (1 MHz, 30 s), and after the following 90 h, the number of dividing cells decreased by 45%. The studies conducted by Ninomiya et al. [205] revealed the high efficiency of the TiO<sub>2</sub> NP-based SDT as a tool to reduce the proliferation of human MCF-7 cells, too. In this case, avidin was immobilized on the surface of TiO<sub>2</sub> NPs in order to enhance its uptake rate by cancer cells. Within 96 h after the termination of the TiO<sub>2</sub> NP sonication (1 MHz, 30 s), the number of viable cancer cells was reduced by 30%.

TiO<sub>2</sub> and ZnO NPs are excellent sensitizers and oxidizing agents. The feature of the ROS generated on their surface is a higher oxidation potential as well as a short half-life (<0.04 µs) [41]. Therefore, the occurrence of ROS-induced oxidative injuries is limited to a small distance (<0.02 µm) from the subcellular position of the sensitizer NPs [54]. NPs administered into the bloodstream become covered by a plasma proteins layer, called protein corona, that lowers their cytotoxicity and, depending on the type of adsorbed plasma proteins, modulates their immunotoxicity and the targeted delivery into tumor cells [206, 207]. Garvas et al. [208] proved the cytotoxicity of TiO<sub>2</sub> NPs in vivo is limited by serum proteins covering their surface that exhibit a trapping ability of photogenerated ROS. The knowledge on the potential application of NPs of TiO<sub>2</sub> and ZnO in therapy and diagnostics of neoplastic maladies is continuously growing. Nonetheless, further studies are necessary to improve the tumor targetability of TiO<sub>2</sub> and ZnO NPs, as well as to increase the therapeutic window of PDT using metal oxide NPs [209, 210].

## Conclusions

Traditional methods of cancer therapy inflict a series of side effects, including the impairment of the immune system. Recent years have witnessed many efforts in creating alternative cancer therapy methods that would be less invasive and would exhibit a site-specific activity. PDT might present a viable solution. Among photosensitizers that have been thoroughly examined in view of their potential application in cancer therapies, there are TiO<sub>2</sub> and ZnO in their NPs form. UV-excited NPs of TiO<sub>2</sub> and ZnO are a source of ROS that form on their surfaces. ROS are agents with a high oxidative potential that damage primarily DNA and cell membranes of cancer cells. Plasma membrane damages lead to cell necrosis, nucleus and mitochondria damages induce apoptotic cell death, and endoplasmic reticulum damages cause cell death by autophagy. Limited tissue penetration ability of UV radiation presents a serious obstacle in the application of UV-sensitizing NPs of TiO<sub>2</sub> and ZnO in cancer therapy. Efforts to apply NIR radiation, since it is less harmful and can penetrate deeper depths tissues, have so far not been successful. Therefore, a new method had to be set up, allowing to generate ROS in deeper depths tissues. Such a method is SDT, using US with low tissue attenuation coefficient. However, SDT exhibits one major disadvantage: insufficient tumor targetability by NPs of TiO<sub>2</sub> and ZnO. The difficulty in preparation of a drug formula that would allow the targeted delivery of NPs to cancer cells is an important factor that restricts the application of photo- and sonosensitizers in cancer therapy. Thanks to the development of nanotechnology, various drug delivery

systems in organisms have been designed. One of them is compiling conjugates of TiO<sub>2</sub> or ZnO in their NPs form with monoclonal antibodies, a method that allows targeting the photo- and sonosensitizers on a selected receptor. The design of modern, more effective cancer fight methods remains an important challenge to contemporary medicine. The scientists are still facing many years of studies on how to optimize cancer therapy using nanotechnology whereas the results have so far been fairly promising.

#### Abbreviations

AMPK: AMP-activated protein kinase; AP-1: Activator protein 1; Apaf-1: Apoptotic protease-activating factor 1; ASK: Apoptosis signal-regulating kinase; ATM: Ataxia telangiectasia mutated kinase; ATR: Ataxia telangiectasia Rad 3-related kinase; BAL: Bronchoalveolar lavage; CASP: Caspase; CAT: Catalase; CB: Conduction band; CLSM: Confocal laser scanning microscope; DDR: DNA damage response; DOPAC: 3,4-Dihydroxyphenylacetic acid; ERK: Extracellular signal-regulated kinase; FA: Folic acid; GPX: Glutathione peroxidase; GSH: Glutathione; ICAD: Inhibitor of caspase-activated DNase; IKK:  $\kappa$ B inhibitor kinase; I $\kappa$ B:  $\kappa$ B inhibitor; JNK: c-Jun N-terminal protein kinase; LPOs: Lipid hydroperoxides; MAPKs: Mitogen-activated protein kinases; MPs: Microparticles; mTOR: Mammalian target of rapamycin kinase; NF- $\kappa$ B: Nuclear factor- $\kappa$ B; NMs: Nanomaterials; NPs: Nanoparticles; NRRT: Neutral red retention time; PDT: Photodynamic therapy; PI3K $\gamma$ : Phosphatidylinositol 3-kinase class III; PKB: Protein kinase B; PMCA: Plasma membrane calcium ATPase; ROS: Reactive oxygen species; SCGE: Single-cell gel electrophoresis; SDT: Sonodynamic therapy; SOD: Superoxide dismutase; Trx: Thioredoxin; US: Ultrasound; UV: Ultraviolet; VB: Valence band

#### Authors' Contributions

JB performed the overall review, drafted the manuscript, and designed all figures. JPC assisted in the research work on the latest developments and perspectives of the application of TiO<sub>2</sub> and ZnO in anticancer therapy and helped draft the manuscript. JZ modified the first version of the draft, prepared its revision, and assisted in the sequence alignment. All authors read and approved the final manuscript.

#### Competing Interests

The authors declare that they have no competing interests.

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