Cerium oxide nanoparticles: Chemical properties, biological effects and potential therapeutic opportunities (Review)

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Abstract. The chemistry of pure cerium oxide (CeO_{2-x}) nanoparticles has been widely studied since the 1970s, especially for chemical catalysis. CeO_{2-x} nanoparticles have been included in an important class of industrial metal oxide nanoparticles and have been attributed a range of wide applications, such as ultraviolet absorbers, gas sensors, polishing agents, cosmetics, consumer products, high-tech devices and fuel cell conductors. Despite these early applications in the field of chemistry, the biological effects of CeO_{2-x} nanoparticles were only explored in the 2000s. Since then, CeO_{2-x} nanoparticles have gained a spot in research related to various diseases, especially the ones in which oxidative stress plays a part. Due to an innate oxidation state variation on their surface, CeO_{2-x} nanoparticles have exhibited redox activities in diseases, such as cancer, acting either as an oxidizing agent, or as an antioxidant. In biological models, CeO_{2-x} nanoparticles have been shown to modulate cancer cell viability and, more recently, cell death pathways. However, a deeper understanding on how the chemical structure of CeO_{2-x} nanoparticles (including nanoparticle size, shape, suspension, agglomeration in the medium used, pH of the medium, type of synthesis and crystallite size) influences the cellular effects observed remains to be elucidated. In the present review, the chemistry of CeO_{2-x} nanoparticles and their impact on biological models and modulation of cell signalling, particularly focusing on oxidative and cell death pathways, were investigated. The deeper understanding of the chemical activity of CeO_{2-x} nanoparticles may provide the rationale for further biomedical applications towards disease treatment and drug delivery purposes.

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1. CeO_{2-x} and CeO_{2-x} nanoparticles

Nanotechnology has become a promising ally for research and treatment of diseases. In addition to a wide range of established applications in multiple areas of research (1), cerium has captured the interest of researchers due to its redox properties. Cerium, a rare earth metal, can exist in both +3 and +4 states. Thus, cerium oxide (CeO_{2-x}) can occur in two different forms: CeO_2 and Ce_2O_3 in the bulk state, due to the coexistence of the element cerium (Ce) in two different oxidation states: Ce^{3+} [(Xe) $4f^1$] and Ce^{4+} [(Xe)]. The CeO_2-Ce_2O_3 phase transition depends on the oxygen pressure and system temperature, as well as the reduction transition (2,3). Among the compounds with Ce^{4+}, the CeO_2 phase presents the most stable structure: A face-centred cubic crystalline network (FCC) of the fluorite type (F*m*3*m*) (4,5). Each tetravalent cerium cation (Ce^{4+}) coordinates with eight oxygen anions (O^{-2}) (Fig. 1A), further providing greater stability compared with the hexagonal structure of Ce_2O_3 (6). The CeO_2 structure could present intrinsic and extrinsic defects in the atom arrangement. Intrinsic defects are related to the thermal disorder of the material or the result of atmosphere surrounding the material, the redox process (7). At the nanoscale, however, the formation of non-stoichiometric oxides is observed in the redox process in CeO_{2-x}, which creates pure CeO_{2-x} nanoparticle structures

where 0 <x <0.5 (8). In this process, electrons are transferred from the oxygen anion (O^{2-}) to the cerium cation (Ce^{4+}), which generates an oxygen vacancy from the reduction of Ce^{4+} to Ce^{3+} (Fig. 1B; Eq. 1).

$$4Ce^{4+} + O^{2-} \rightarrow 4Ce^{4+} + 2e^{-} + ø + 0.5O_2 \rightarrow 2Ce^{4+} + 2Ce^{3+} + ø + 0.5O_2$$

(Eq. 1: ø represents the empty position due to the atom displacement in the structure after electron transfer).

An oxygen vacancy is a structural disarrangement caused by the increase or decrease of oxygen concentration inside the particle and cerium ion radius. It is important to note that the concentration of oxygen defects increase with the reduction in particle size, which confers better redox properties of CeO_{2-x} nanoparticles compared with their oxide (9,10). In chemical catalysis, vacancy is defined as the ability of an oxide to store and release oxygen. The description of oxygen vacancies in transition oxides and rare earth oxides is an unexplored challenge for modern calculations of electronic structure (11). Due to the increase in the surface-to-volume ratio, CeO_{2-x} nanoparticles have higher concentrations of Ce^{3+}, compared with CeO_{2-x} particles. Thus, there is greater mobility of oxygen in the structure for the rapid generation of surface vacancies. This property provides easy switching between Ce^{3+} and Ce^{4+} oxidation states, generating numerous active points for redox reactions to occur on the surface of nanoparticles. Due to their oxygen buffering capacity, CeO_{2-x} nanoparticles can also self-regenerate to the initial state of Ce^{4+} with no side reactions (12).

Nanoparticle structural defects-vacancies. Vacancies are structural defects in a particle, which can be formed through electronic relocation in the structure of the material, generating redox hotspots. Vacancies are not restricted to the oxide surface only. For CeO_{2-x}, studies demonstrate that the electrons resulting from the formation of oxygen vacancies on the surface and subsurface in the crystallographic plane (111; Miller index) may not go to the atoms directly linked to the electron donator atom, but to more distant atoms instead (11,13). Due to the increase in the surface area, CeO_{2-x} nanoparticles have more Ce^{3+} ions on their surface and their vacancies can occur by a quantum ionization/displacement process of cerium 4f electrons, as proposed by Skorodumova *et al* (14). Electrons in the *f* subshell create redox hotspots by the reduction of Ce^{4+} to Ce^{3+} (11). In addition, the migration of electrons from 2p orbital oxygen states in the valence to the conduction band is only possible if the energy between them (band gap) is relatively small (14,15). In CeO_{2-x} nanoparticles, vacancies are dynamic and can change spontaneously or in response to physical parameters, such as temperature, partial pressure of oxygen, doping with other ions and application of an electric field or surface stress (16,17). Another phenomenon responsible for causing vacancies is entropic stabilization, which will appear on surfaces with numerous empty spaces. Beyond particle size, some other factors may influence the redox activity of the nanoparticles, such as suspension medium and formation of agglomerates. Reducible oxide surfaces, such as CeO_{2-x} nanoparticles, are highly disordered at the nanoscale, causing a greater formation of empty spaces, facilitating the formation of vacancies, and presenting even greater redox activity (18). Due to their high redox activity, CeO_{2-x} nanoparticles have started to gain attention in the biomedical field, particularly with regard to diseases in which a redox imbalance is observed. It is important to note that a redox imbalance can be found in a wide range of diseases, including cancer (19). Therefore, some studies have addressed the oxidative stress-associated cytotoxic effects of CeO_{2-x} nanoparticles in biological models, particularly focusing on the modulation of the apoptotic pathway, the most studied regulated cell death modality (Tables I and II). This is relevant, considering the increasing body of evidence characterizing genetically-regulated distinct mechanisms of cell death (20). Due to the wide diversity of cell death subroutines, the present study focuses only on the effects of nanoparticles on apoptotic cell death.

2. CeO_{2-x} nanoparticles mimic antioxidant enzymes

Due to their physical and chemical redox properties, CeO_{2-x} nanoparticles have begun to be used in the biomedical field, with the aim of restoring normal tissue homeostasis. CeO_{2-x} nanoparticles exhibit controversial pro-oxidant and antioxidant activity, which enable them to react with chemical elements such as oxygen, nitrogen, sulphur and chloride (21). It has previously been described that CeO_{2-x} nanoparticles have the ability to mimic some enzymes, such as catalase (CAT) and superoxide dismutase (SOD), and neutralise reactive oxygen species (ROS) (21).

Korsvik *et al* (22) have described CeO_{2-x} nanoparticles exhibiting SOD-like activity, hypothesizing that they confer cellular protection. These findings revealed that the surface oxidation state of CeO_{2-x} nanoparticles plays an essential role in the SOD mimetic activity, found to be dependent on the concentration of the +3 oxidation state (22). It suggests a positive association between the trivalent oxidation state of CeO_{2-x} nanoparticles and the mimetic activity of SOD (22,23). A single nanoparticle has been shown to be more efficient as a SOD catalyst than the natural SOD enzyme, with a catalytic rate of $3.6x10^9$ $M^{-1}sec^{-1}$ compared with 1.3 and $2.8x10^9$ $M^{-1}sec^{-1}$ of a natural SOD enzyme (22). In contrast to results attributing a SOD mimetic activity for CeO_{2-x} nanoparticles, experiments conducted by Pirmohamed *et al* (24) showed CeO_{2-x} nanoparticles acting as CAT mimetics in a redox state-dependent manner. Their findings demonstrated that only CeO_{2-x} nanoparticles with fewer surface cerium atoms in the +3 state exhibited any significant CAT mimetic activity. These findings are especially noteworthy, since CeO_{2-x} nanoparticles with lower +3/+4 ratios were revealed to be less efficient in their SOD mimetic activity, and thus there appears to be an inverse realtionship between catalysis and the cerium oxidation state of the nanoparticle (23).

It should be noted that the effects of SOD and CAT mimetics can be enhanced or suppressed by CeO_{2-x} nanoparticle surface modifications. Yadav and Singh (23) demonstrated that CeO_{2-x} nanoparticles coated with phosphotungstic acid (PTA) exhibited an enhanced SOD and CAT mimetic activity independent of the majority of nanoparticle surface charges (+3 or +4). When covering the nanoparticle with phosphomolybdic acid (PMA), SOD activity was suppressed in CeO_{2-x} nanoparticles with its +3 surface charge, however no effect was observed on CAT activity. In addition, PMA covering exerted no effect on SOD while CAT acitvity was enhanced in CeO_{2-x}

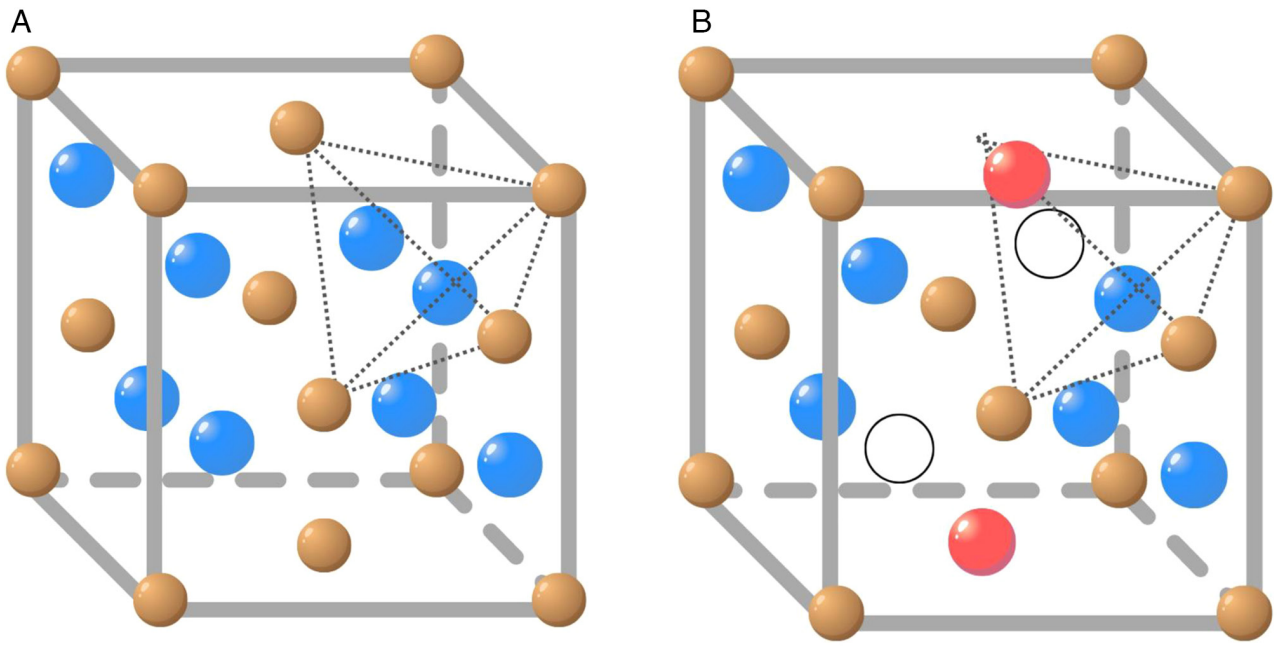

Figure 1. Schematic figure of (A) unit cell of cerium oxide with a face-centered cubic crystalline network structure of the fluorite type and (B) formation of non-stoichiometric oxide during the redox process. Ce^{4+} is represented by brown balls, O^{2-} by blue balls, Ce^{3+} by red balls and the vacancies, by the empty circles.

nanoparticles with a +4 surface charge. PTA and PMA are both electron-dense molecules and display quick and reversible multielectron redox reactions, a probable explanation for the surface charge-dependent effects on SOD/CAT (23).

Notably, a molecule exhibiting the same characteristics as PTA and PMA was demonstrated to exert the opposite effects from those aforementioned. Triethyl phosphite (TEP) altered the SOD and CAT mimetic activities of CeO_{2-x} nanoparticles. A higher Ce^{4+}/Ce^{3+} surface oxidation state exerted a decrease in CAT mimetic activity while SOD activity was increased. In addition, in this study a correlation between TEP concentration and the formation of surface oxygen vacancies was reported (25).

Of note, PMA, PTA and TEP are molecules that contain phosphorus. For example, Karakoti *et al* (26) described that CeO_{2-x} nanoparticles coated with the polymer polyethylene glycol (PEG), which does not hold phosphorus in its composition, did not affect the SOD mimicking activity of the nanoparticle. In view of the results herein mentioned, CeO_{2-x} nanoparticles exhibit SOD and CAT mimetic activity, and their effects may be enhanced or suppressed according to which type of molecules cover the the surfaces of the nanoparticles. Further studies analysing the influence of different chemical functional groups on SOD and CAT mimetic activities on the surface of nanoparticles are required.

3. Biological effects of CeO_{2-x} nanoparticles in disease models with dysregulated apoptosis and redox imbalance

Apoptosis is the most studied cell death modality and can be triggered by a wide range of stimuli, such as DNA damage, nutrient deprivation, hypoxia, viral infections, growth factor and hormone signalling. In addition, apoptosis involves the activation of two main biochemical pathways (intrinsic and extrinsic) and leads to various events such as cell retraction, bleb formation, protein cleavage, DNA degradation, and phagocyte recognition (20). Apoptosis dysregulation is involved in the pathogenesis of numerous diseases, including progressive cochlear and retinal degeneration (27), liver fibrosis (28), hypoxia in brain cells (29), type 1 diabetes (30), neurodegenerative diseases (31) and cancer (32). For some of these conditions, apoptosis is defective, and thus cells are capable of surviving even upon cell death stimuli. For others, apoptosis is aberrant, and is associated with organ degeneration and failure. Restoring apoptosis is essential for tissue homeostasis and great effort has been devoted to inducing or inhibiting this form of cell death, depending on the cellular and disease context. Although the scope of this manuscript is apoptosis, the increasingly significant role of other regulated cell death modalities directly modified by cellular oxidative stress, such as ferroptosis or paraptosis, cannot be excluded (20).

Furthermore, the accumulation of ROS has been described to be associated with the development of pathologies such as diabetes, atherosclerosis, stroke, arthrosis, amyotrophic lateral sclerosis, neurodegenerative disorders including Parkinson's and Alzheimer's diseases and cancer (10). ROS play a central role in cell signaling as well as regulation of the main pathways of apoptosis mediated by mitochondria, death receptors and the endoplasmic reticulum (ER) (33-35). Notably, several cytotoxic agents have been shown to induce apoptosis by ROS production (36-39). Nevertheless, low levels of ROS play an important role in the signal transduction process inside the cell, acting as second messengers in the physiological environment. Therefore, it is important to finely tune the balance between cellular oxidative stressors and the antioxidative defence to maintain homeostasis and impair pathological states (10).

In this context, with the increasing interest in the redox characteristics of CeO_{2-x} nanoparticles, numerous authors have

Table I. Effects of cerium oxide nanoparticles in non-cancer disease models.

Disease models	Redox activity/ Apoptosis regulation	Nanoparticle characteristics	(Refs.)
Alveolar epithelial cells	Antioxidant/Oxidant	Not described	Lord *et al* (21)
Progressive cochlear and retinal degeneration in Tubby mice	Antioxidant/Apoptosis inhibition	Medium, saline	Kong *et al* (40)
Hepatic fibrosis	Antioxidant/Apoptosis inhibition	Synthesis, chemical precipitation; Size, 4-20 nm; medium, aqueous solution of TMAOH	Oró *et al* (41)
Type 1 diabetes	Antioxidant/Apoptosis inhibition	Purchased from Sigma-Aldrich; size, 90 nm; shape, cubes	Khurana *et al* (42)
Activated and non-activated human monocytic cells	Antioxidant/Oxidant	Synthesis, flame spray pyrolysis; size: 3-94 nm	Schwotzer *et al* (43)

TMAOH, tetramethylammonium hydroxide; PBS, phosphate saline buffer.

Table II. Effects of cerium oxide nanoparticles in cancer cellular models.

Cancer model	Redox activity/Apoptosis regulation	Nanoparticle characteristics	(Refs.)
Breast cancer and healthy breast cells	No effect in breast cancer cells and antioxidant in healthy breast cells	Synthesis, microemulsion process; size, 3-5 nm	Tarnuzzer *et al* (50)
Lung carcinoma, melanoma and colorectal adenocarcinoma and healthy (origin) cells	Oxidant in tumors/No apoptosis induction	Synthesis, SPRT; shape, cubic; size: 4 nm	Pešić *et al* (62)
Alveolar adenocarcinoma, hepatoma, colorectal cancer, cervical cancer and healthy (origin) cells	Antioxidant	Purchased from Sigma-Aldrich; size, <25 nm; medium, DMEM	Rubio *et al* (57)
Hepatoma	Oxidant	Shape, hexahedral; size, 20-30 nm	Cheng *et al* (58)
Hepatoma	Unchanged/Apoptosis inhibition	Not described	Cheng *et al* (61)
Hepatoma	Apoptosis induction	Synthesis, chemical precipitation; size, 4-5 nm; medium, aqueous solution of TMAOH	Fernández-Varo *et al* (60)
Melanoma	Oxidant/Apoptosis induction	Purchased from Sigma-Aldrich; size, 20-40 nm	Ali *et al* (53)
Melanoma	Oxidant/Apoptosis induction	Purchased from Sciventions; Medium, water	Aplak *et al* (63)
Colorectal carcinoma	Oxidant/Apoptosis induction	Synthesis, chemical precipitation; size, 30-40 nm	Datta *et al* (64)

SPRT, self-propagating room temperature method; TMAOH, tetramethylammonium hydroxide.

dedicated studies to their role in modulating apoptotic signalling pathways in a wide range of disease models.

Effects of CeO_{2-x} nanoparticles in disease models exhibiting excessive apoptosis. Some studies have shown CeO_{2-x} nanoparticles as negatively regulating apoptosis pathways in animal models of diseases that exhibit aberrant apoptosis (Table I; Fig. 2). For example, in an *in vivo* model of cochlear and retinal degeneration, CeO_{2-x} nanoparticles were shown to upregulate, at the protein level, the basic fibroblast growth

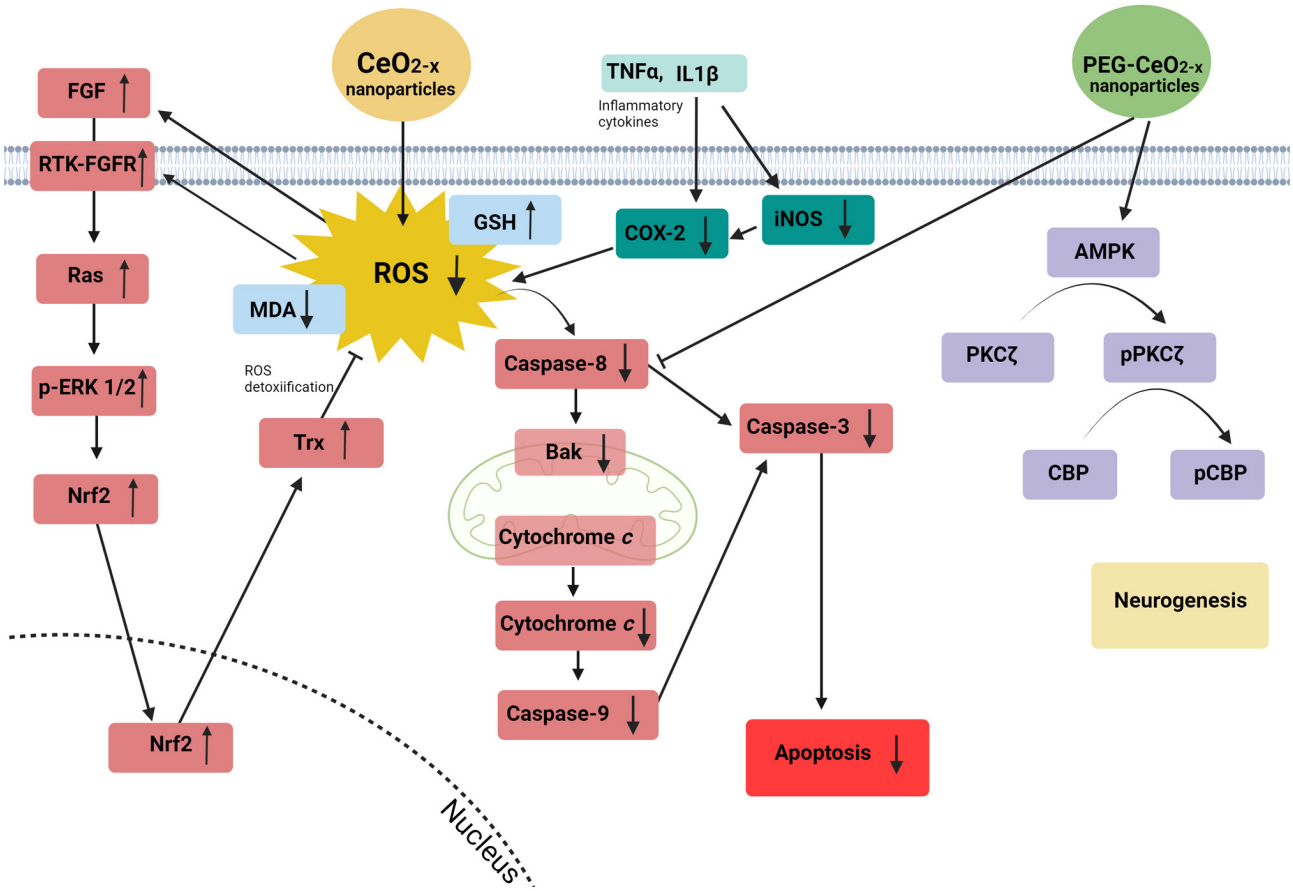

Figure 2. Cellular signalling pathways affected by CeO_{2-x} nanoparticles in disease models with excessive apoptosis. In an *in vivo* model of cochlear and retinal degeneration (40). CeO_{2-x} nanoparticles stimulate the bFGF/RTK/Ras/ERK pathway by reducing ROS. Under this stimulus, the Ras-p-ERK cascade activates Nrf2, which then increases the expression of Trx, further inhibiting the production of ROS. Reduced ROS levels lead to a decrease in the activity of caspases 3, 8 and 9, Bak-1 expression and release of cytochrome *c* from the mitochondria, inhibiting the apoptosis process. In liver fibrosis, CeO_{2-x} nanoparticles reduce the levels of TNFα, IL1β, COX-2 and iNOS pro-inflammatory cytokines (41). In type 1 diabetes, CeO_{2-x} nanoparticles exhibit a significant decrease in the levels of MDA and an increase in GSH, which may suggest lower ROS levels after treatment with CeO_{2-x} nanoparticles (42). In hypoxic brain cells, CeO_{2-x} nanoparticles coated with polyetilenoglycol (PEG-CeO_{2-x}) act on the AMPK/PKCζ/p-PKCζ/CBP/p-CBP pathway and inhibit caspase-8, leading to neurogenesis and inhibition of the apoptotic process (29). CeO_{2-x}, cerium oxide; FGF, fibroblast growth factor; RTK, receptor tyrosine kinase; ERK, extracellular signal-regulated kinase; ROS, reactive oxygen species; p-, phosphorylated; Nrf2, nuclear factor 2; Trx, thioredoxin; Bak-1, BCL-1-antagonist-killer; TNFα, tumor necrosis factor α; IL1β, interleukin-1β; COX-2, cyclooxygenase-2; iNOS, inducible nitric oxide synthase; MDA, malondialdehyde; GSH, glutathione; AMPK, 5' AMP-activated protein kinase; PKCζ, protein kinase Cζ; CBP, CREB-binding protein.

factor (bFGF)/receptor tyrosine kinase (RTK)/Ras/extracellular signal-regulated kinase (ERK) pathway, indicated as essential for cell proliferation and survival (40). An increase in the protein expression of thioredoxin (Trx), nuclear factor 2 (Nrf-2), and nuclear Nrf-2 antioxidant proteins and a decrease in the ROS concentration and in the mRNA levels of caspase 8 and BCL-1-antagonist-killer (Bak-1) was also revealed in this study. In addition, a decrease in the catalytic activity of caspases 3 and 9 and improved release of cytochrome *c* from the mitochondria were reported (40). These findings clearly attribute an antioxidant activity to CeO_{2-x} nanoparticles, at least in the model in context.

For liver fibrosis, a chronic liver disease characterized by excessive apoptosis (28), Oró *et al* (41) demonstrated that CeO_{2-x} nanoparticles reduced steatosis (accumulation of fat in the liver), portal hypertension (abnormal increase in blood pressure in the portal vein that transposes blood from the intestine to the liver) and the levels of hepatic pro-inflammatory cytokines in rats, thereby attenuating the inflammatory response. Additionally, a marked reduction in the mRNA expression of inflammatory cytokines [tumor necrosis factor α (TNFα), interleukin-1β (IL1β), cyclooxygenase-2 (COX-2) and inducible nitric oxide synthase (iNOS)], endothelin 1 (ET-1) and messengers related to the oxidative stress signaling pathway [eosinophil peroxidase precursor (Epx), neutrophil cytosolic factor 1 and 2 (Ncf1 and Ncf2)] or endoplasmic reticulum [cyclic AMP-dependent transcription factor (Atf3) and heat shock protein family A (Hsp70) member 5 (Hspa5)] was observed. This was associated with reduced macrophage infiltration and reduced abundance of active caspase-3 protein, immunostaining of α-smooth muscle actin (α-SMA) and both protein and gene expression of inflammatory cytokines.

An *in vivo* study conducted by Khurana *et al* (42) for type 1 diabetes induced in Swiss mice, revealed a significant decrease in malondialdehyde (MDA), a ROS marker, and nitric oxide levels and an increase in glutathione (GSH; part of antioxidant defence) levels and insulin production following CeO_{2-x} nanoparticle treatment. In CeO_{2-x} nanoparticle-treated mice, the levels of intracellular SOD increased while the expression of caspase-3 and DNA damage decreased. Collectively, these

findings indicate that CeO_{2-x} nanoparticles act as antioxidant agents and modulate signalling pathways (Fig. 2), particularly those resulting in apoptosis inhibition, in the context of diseases with excessive apoptotic levels.

Conversely, some studies have shown CeO_{2-x} nanoparticles acting as an oxidant agent generating inflammatory and oxidative stress in models exhibiting defective apoptosis, further inducing positively the apoptotic pathway. As an example, a previous study performed by Schwotzer *et al* (43), revealed CeO_{2-x} nanoparticles acting as inflammatory and oxidative stress agents in *in vitro* tests in alveolar epithelial cells, verified through the release of chemokines and characterized by the release of increased monocyte activation with subsequent neutrophil and lymphocyte infiltrations. These findings, although in contrast to the previous ones aforementioned, show the duality of CeO_{2-x} nanoparticles and a possible role in the inflammatory system, which remains to be investigated. In cancer, in which cell death is defective (44) mainly due to dysregulation of Bcl-2 family members (45) and inhibitor of apoptosis proteins (46), CeO_{2-x} nanoparticles can also act as an oxidative agent. In the present study, an overview of the effects of CeO_{2-x} nanoparticles in cancer models, from the first published studies to the analysis of how CeO_{2-x} nanoparticles modulate the apoptotic pathway is presented.

Effects of CeO_{2-x} nanoparticles in models exhibiting defective apoptosis: CeO_{2-x} nanoparticles and cancer. It is well known that the development and progression of cancer are associated with adaptation to oxidative stress and dysregulation of the expression of antioxidant enzymes (47,48). In addition, ROS can act as messengers in signal transduction and induce DNA damage, further leading to carcinogenic lesions (49). As described in the previous section, CeO_{2-x} nanoparticles have been shown to be potent scavengers of certain free radicals and may then be potentially effective in diseases exhibiting high levels of ROS production. However, CeO_{2-x} nanoparticles have also been demonstrated to be either protective or induce oxidative stress in cancer, indicating the diversity of the biological effects of CeO_{2-x} nanoparticles in this scenario.

Radioprotective and sensitizing effects of CeO_{2-x} nanoparticles. The first study described in literature assessing the effects of CeO_{2-x} nanoparticles on tumour lines was carried out by Tarnuzzer *et al* (50), who treated breast carcinoma tumour cells (MCF-7) and breast epithelial cells (CRL8798) with CeO_{2-x} nanoparticles and analysed their response to radiation. While healthy cells were protected by nanoparticles (showing no cytotoxicity), breast tumour cells were more sensitive to radiation. The radioprotective capacity of CeO_{2-x} nanoparticles in healthy epithelial cells could be possibly explained by their antioxidant activity attributed to the chemical characteristic of its self-regenerating redox activity. Conversely, most solid tumours have an acidic microenvironment due to the glycolytic metabolic pathway and exacerbated lactate production (51). Tumour acidosis may then disable the antioxidant activity of the nanoparticle, potentiating their oxidizing activity and, consequently, sensitizing the tumour to radiation therapy (52).

Effect of pH on CeO_{2-x} nanoparticle activity. Variations in pH are important factors affecting CeO_{2-x} nanoparticle activity. In general, neutral pH promotes cytoprotective effects, while acidic pH leads to cytotoxic effects (52). Due to its redox behaviour, CeO_{2-x} nanoparticles can mimic some enzymes such as as SOD, CAT and oxidases (50). When CeO_{2-x} nanoparticles mimic SOD, the dismutation of $O_2^{\bullet-}$ and generation of H_2O_2 and then, O_2 is observed. In this case, a higher Ce^{3+} surface concentration leads the nanoparticles to exert the same mechanism of action as SOD. On the flip side, a higher surface concentration of Ce^{4+} leads the nanoparticles to mimic CATs, degrading H_2O_2. In an acidic pH, CAT properties decrease significantly, but SOD properties remain the same. A single CeO_{2-x} nanoparticle has been revealed to be more efficient as a SOD catalyst than the natural enzyme (22,53). Some studies have shown that the CAT-like scavenger activity of CeO_{2-x} nanoparticles is inhibited in an acidic pH environment. Notably, while the rate of superoxide conversion to peroxide is not affected by pH variations, CeO_{2-x} nanoparticles cannot detoxify the hydrogen peroxide at the same rate in an acidic pH. Therefore, CeO_{2-x} nanoparticles could be harmful in a low pH environment (54-56).

A study by Rubio *et al* (57) identified antioxidant activity of CeO_{2-x} nanoparticles in both human tumoral cell lines and mouse embryonic fibroblast (MEF) non-neoplastic cells. In this study, the influence of pH on the action of CeO_{2-x} nanoparticles in the A549 human lung alveolar adenocarcinoma cell line was assessed, but it was found that the antioxidant properties of CeO_{2-x} nanoparticles were not influenced.

Modulation of the apoptotic pathway by CeO_{2-x} nanoparticles in cancer. A study conducted by Cheng *et al* (58) revealed that CeO_{2-x} nanoparticles were effective in inducing apoptosis in the SMMC-7721 human hepatoma cell line. In this study, CeO_{2-x} nanoparticles had a non-spherical hexahedral shape between 20-30 nm. After treatment with CeO_{2-x} nanoparticles, increased levels of ROS and MDA marker, and decreased levels of antioxidant SOD, GSH-px and CAT were found (Fig. 3). In addition, there was an increase in the expression of phosphorylated (p)-p38, p-c-Jun N-terminal kinase (JNK) and p-ERK1/2, followed by improved apoptosis rates. According to a study by Xia *et al* (59), CeO_{2-x} nanoparticles can induce spontaneous ROS production inducing a protective response. This is likely since cellular responses such as inflammation or mitochondrial damage also act as injury response pathways to stressors other than oxidative stress. Upon adaptation, cells can activate the SOD, GSH and CAT antioxidant enzymes.

Accordingly, a previous study by Fernández-Varo *et al* (60) demonstrated that hepatoma cells treated with CeO_{2-x} nanoparticles had an activation of caspase-3, increased expression of ERK1/2 and a decrease in the expression of p-ERK-1/2. Collectively, the results from Cheng *et al* (58) and Fernández-Varo *et al* (60) indicate that CeO_{2-x} nanoparticles may activate distinct signalling pathways when triggering apoptosis cell death in hepatoma *in vitro* models. Conversely, PEG-coated CeO_{2-x} nanoparticles conjugated with alendronate (specific bone resorption inhibitory drug) were found to have protective effects on hepatoma cells, marked by an increased in the ratio of Bcl-2/Bax and p-protein kinase B (p-AKT) and p-ERK, with no change in the levels of ROS (61). In addition, tumour size *in vivo* increased after CeO_{2-x} nanoparticle treatment, further confirming that CeO_{2-x} nanoparticle-PEG-alendronate promotes cell proliferation and should not be considered for potential therapeutic anticancer purposes.

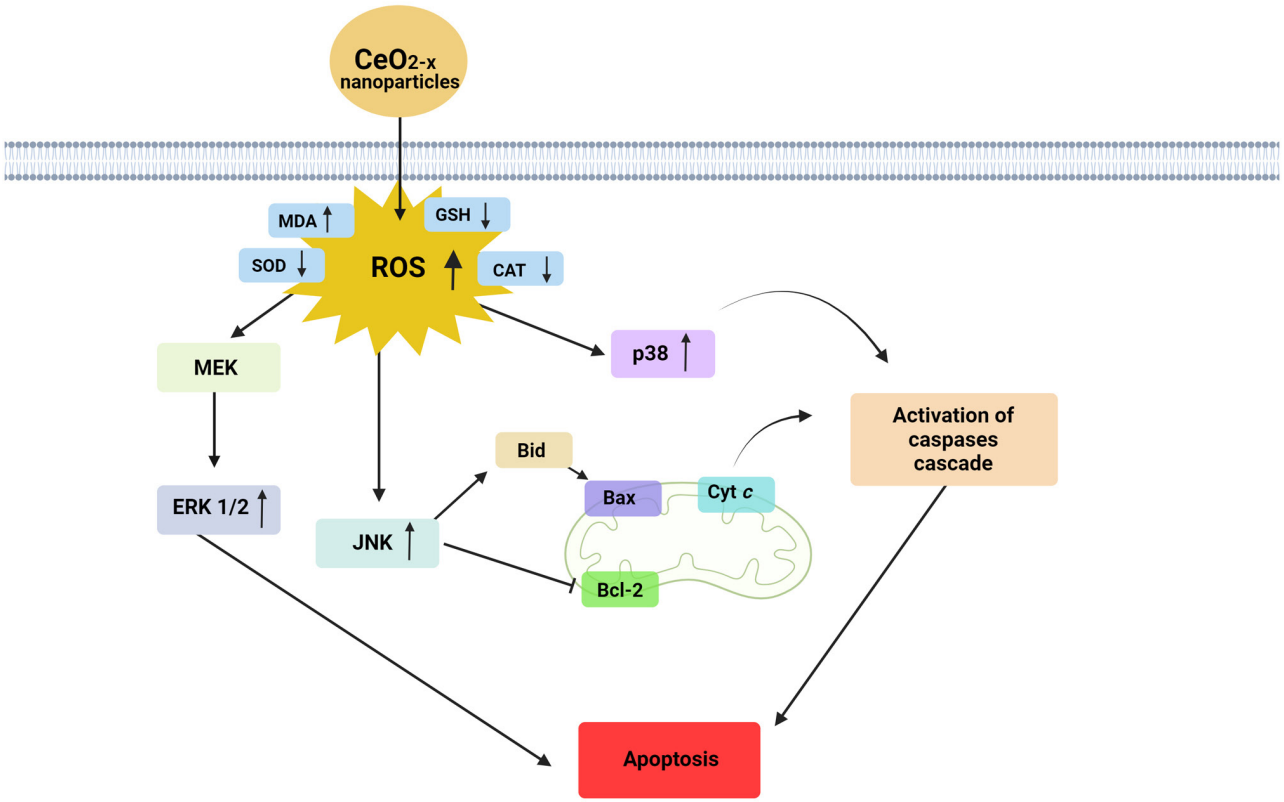

Figure 3. Cellular signalling pathways affected by CeO_{2-x} nanoparticles in cancer. For human hepatomas, CeO_{2-x} nanoparticles decreased ROS concentration, as shown by the increase in the levels of MDA and decrease in the levels of GSH (58). Under ROS stimuli, expression of MEK/ERK, JNK, p38 and caspase-3 was also increased, leading to activation of an apoptotic cascade. JNK acts on the regulation of Bid and on the inhibition of Bcl-2, affecting the mitochondrial membrane potential and leading to the activation of the apoptotic pathway via caspases. In human melanoma models, CeO_{2-x} nanoparticle treatment promoted an increase in ROS and MDA, but a decrease in GSH, SOD and CAT levels (61). For colorectal carcinoma models, the induction of apoptosis through activation of caspase-3 and caspase-9, release of cytochrome *c* in the cytoplasm, high ROS levels and disrupted membrane potential (64) is observed. CeO_{2-x}, cerium oxide; ROS, reactive oxygen species; MDA, malondialdehyde; GSH, glutathione; MEK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; SOD, superoxide dismutase; CAT, catalase.

In human melanoma models, CeO_{2-x} nanoparticles were revealed to have impaired cell viability in a dose-dependent-manner, decreased levels of GSH and increased levels of caspase-3, ROS, MDA and SOD. In addition, there was an increase in double strand DNA breaks following ROS generation, suggesting that CeO_{2-x} may promote oxidative stress-mediated apoptosis and DNA damage (63). Furthermore, in melanoma, Aplak *et al* (63) found that CeO_{2-x} nanoparticles could induce the levels of mitochondrial ROS, accompanied by an increase in the oxidation of mitochondrial thiol and promotion of hydrogen peroxide-linked mitochondrial dysfunctions. The cytotoxic effects of CeO_{2-x} nanoparticles have also been demonstrated in colorectal carcinoma models, in which a dose-dependent induction of apoptosis was found. This was associated with activation of caspase-3 and caspase-9, release of cytochrome *c* in the cytoplasm, high ROS levels and disrupted membrane potential (64). Taken together, most preclinical studies indicate that CeO_{2-x} nanoparticles exhibit cytotoxic activity in experimental models of cancers from different origins, which may encourage future work with animal models.

4. Concluding remarks and future perspectives

With the increasing interest in the dual characteristics of CeO_{2-x} nanoparticles, as either an oxidant or antioxidant, numerous authors have begun to investigate their participation in apoptosis signalling pathways. In some cases (as for example, in cancer), its induction would be favourable to help control the disease. In other cases (such as in liver fibrosis), its inhibition would be ideal to interrupt the disease-associated degenerative features.

Apoptosis can be triggered by different factors and several pathways can be activated to promote cell termination. Notably, CeO_{2-x} nanoparticles can modulate some of these pathways, which could be particularly useful for future cancer treatments (44). Although this review highlights the effects of CeO_{2-x} nanoparticles in modulating apoptotic cell death, other forms of cell death have also been shown to be triggered by these nanoparticles (65-67). The cytotoxic effects of CeO_{2-x} nanoparticles in non-neoplastic cells are minor, some even cytoprotective against cytotoxic stimuli.

Considering the dual behaviour of CeO_{2-x} nanoparticles as either antioxidant or pro-oxidant (oxidation no. change between +3 → +4 → +3), it is important to mention that nanoparticle action may not only be influenced by tumour features, but also by its structural and chemical characteristics. It is most likely that the effects of CeO_{2-x} nanoparticles are dependent upon the tumour origin and oxidative state, the subcellular compartment where it is located within cells and how acidic the tumour microenvironment is.

Last but not least, it should be noted that numerous factors such as particle shape, size and structure as well as the solvents used to prepare them for experimental cellular assays may all affect the properties of CeO_{2-x} nanoparticles. Unfortunately, this piece of information is most often overlooked by studies, which lack discussion on the subject. In general, studies have been carried out with nanoparticles of different sizes, synthesis routes and procedures used to prepare their suspension for biological assays. For a better understanding of the effects of CeO_{2-x} nanoparticles in a biological environment, a more detailed description of the chemical structure and properties of nanoparticles must be elucidated (43,54). Combining information from both chemical and biological models is key to address the potential future role of CeO_{2-x} nanoparticles and other nanoparticles in therapeutics and further biomedical applications.

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Availability of data and materials

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Authors' contributions

All authors (MBDSA, GNDM and KRDS) designed and conceived the study, performed the research, and wrote and edited the manuscript. Data authentication is not applicable. All authors have read and agreed to the published version of the manuscript.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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