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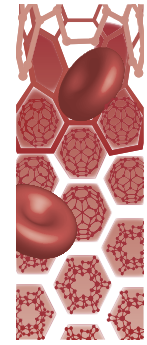

Cerium oxide nanoparticles and their importance in cell signaling pathways for predicting cellular behavior

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Cerium oxide nanoparticles (CeO_2-NPs) have prolifically attracted immense interest of researchers due to their prominent anti-oxidant nature. However, these characteristics are accompanied by some ambiguities in other studies reporting their oxidant and toxic properties. In this regard previous literature has pointed to the importance of the NPs morphology and environmental conditions as well as biomolecules that induce a different response by initiating a cascade of activities. Therefore, due to the fact that signaling proteins are key mediators in cellular responses, the cognizance of the CeO_2-NP-targeted signaling pathways could facilitate predicting the cellular behavior and thus more efficient applications of these NPs for clinical purposes. Consequently, a comprehensive review is necessary in this field, to clarify the impacts of CeO_2-NPs on various signaling pathways.

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Cerium oxide nanoparticles (CeO_2-NPs) have received considerable attention in various fields including engineering, biology and regenerative medicine. Switching between Ce^{3+} and Ce^{4+} states changes oxygen vacancy concentrations in CeO_2-NPs crystal structure and subsequently their potentials for scavenging free radicals such as reactive oxygen species (ROS) [1]. Free radicals are natural products of aerobic metabolism and act as signal mediators which can trigger various cellular responses. Nevertheless, high levels of ROS could result in oxidative stress causing damage to DNA, proteins and lipids [2]. So far, many articles have shown that CeO_2-NPs with anti-oxidant [3] and anti-inflammatory [4,5] properties could be exploited in nanotherapeutics to provide targeted drug delivery or combinatory treatments [6–9] and improve diseases related to ROS such as cancers [10], neurological disorders [11], autoimmune degenerative diseases [12], cardiovascular disease [13] – among others. Applications of CeO_2-NPs in tissue engineering [14], radiation protection and wound healing [15] have also been reported. It is worth noting that despite many exciting therapeutic properties of CeO_2-NPs, there have been conflicting results concerning their functions as both pro- and anti-oxidant agents. For example, Ma *et al.* [16] showed that CeO_2-NP-induced inflammation can lead to lung fibrosis; while, some literatures indicate that CeO_2-NPs not only have no toxicity on the lungs in the optimized conditions, but also play a protective role against oxidative stress and inflammation [17,18]. On the other hand, it has been reported that use of CeO_2-NPs resulted in elevated ROS and TNFα levels, decreased superoxide dismutase (SOD) activity and induced DNA damage in various organs [19]. There are also other studies which have shown toxic effects of CeO_2-NPs on biological systems [20,21]. So far, extensive studies have been done to understand the cause of these inconsistencies and expressed various reasons including particle size, shape, concentration and dosing schedule [22], ions, organic acid and polymers, pH, oxygen levels and redox agents in liquid phases [23] as well as the formation of a protein corona and aggregation into micro-size clusters due to different behaviors of these particles in biological fluids [24]. Some literatures have also studied how cells

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respond to these nanoparticles and considered the mechanisms of cellular absorption and intracellular localization as important factors in the behavior of CeO_2-NP-exposed cells [25]. However, the results of these articles are also controversial. Although, it seems that these contradictions could cause many limitations, on the other hand, it has been demonstrated that these nanoparticles can have design flexibility for using in different nanomedicine applications. Therefore, it is necessary to reach a comprehensive understanding of the interactions between CeO_2-NPs redox performance, their physicochemical properties and microenvironmental conditions as well as components of targeted-living system for better predicting implications of CeO_2-NPs reactivity and the response of exposed cells. Some studies also represented solutions to stabilize NPs by controlled conditions including encapsulation [26,27], conjugation [28–30] and coating [24]. Kumar *et al.* comprehensively studied current challenges of CeO_2-NPs in nanobiomedicine applications and indicated the requirement to fill in the current gaps [31]. In addition, currently, systems biology has developed as an interdisciplinary science that applies in a broad range of fields [32]. This novel approach employs computational modeling, bioinformatics tools and quantitative molecular biology techniques (omics) to study the complex interactions between different components of a biological system to predict the system's behavior [33]. In the context of nanotoxicology, these approaches analyze the suggested hypotheses for the mechanisms of cell response to nanomaterials and ultimately propose how the cell and consequently the system will behave.

As pointed above, computational modeling or simulation accelerates checking the accuracy of a proposed hypothesis, reduces the number of difficult, expensive and long-term tests in the laboratory, recognizes gaps in biological system networks and predicts studied-systems behaviors [34]. It is important to highlight that the first and fundamental step in modeling is system delimitation and acceptable knowledge requirement of targeted biological systems. In this regard, some studies utilized these basic data and mathematical sciences, in order to achieve comprehensive information about how redox activity of CeO_2-NPs changes in different biological systems. Reed *et al.* [35] presented a mathematical model to predict ROS-CeO_2-NPs interactions and suggested methods for optimizing experimental studies. In addition, they accurately examined the data difference or alignment of their simulated models, in both *in vitro* and *in vivo* conditions and ultimately indicated potential ROS modulation, SOD mimetic, catalytic regeneration, self-regulation and self-limiting behaviors of CeO_2-NPs [35]. The distribution of CeO_2-NPs in various tissues and organs of rats was also modeled by Carlander *et al.* based on experimental information collected from published literatures [36]. This simulation confirmed associations between CeO_2-NPs accumulation and their physical properties, exposure methods and applied dose [36].

Although many efforts have been made to resolve these ambiguities related to CeO_2-NPs applications or functions, as explained above, it should be noted that in the living systems the signaling pathways sense, amplify and integrate various external signals to change biological activity of target cells. So, these are the main agents in the critical cellular processes such as proliferation, differentiation, apoptosis – among others. Moreover, it is important to emphasize that cell decisions and fate have been determined via these signal transduction pathways. Consequently, knowledge of these mediators which process external stimuli underlies the analysis and prediction of the cellular behavior. Increasing and widespread applications of CeO_2-NP and the ambiguous evidences linking its functional potential require an immediate need for understanding CeO_2-NP-targeted signaling pathways.

Thus, in summary, the adequate knowledge of signaling pathways as the core of many critical cellular functions is important and necessary to answer various questions including how CeO_2-NPs change cellular behaviors or which signaling pathways are involved, whether CeO_2-NPs modulate directly a signaling pathway via regulation of its related gene expression or indirectly via controlling ROS level and so on.

In this review, we discuss the underlying molecular mechanisms in the previous studies, in order to evaluate the efficiency or risk assessment of CeO_2-NPs in clinic. We focus on the importance of an often-neglected aspect – the CeO_2-NP-targeted signaling pathways – which could promote different cellular functions. So, we present general information about CeO_2-NP-targeted signaling pathways as an important starting point so that researchers consider the complexity of the CeO_2-NP-cell signaling pathways before designing any experiments.

Antioxidant pathways

Oxidative stress refers to an imbalance between the production and elimination of ROS that acts like a double-edged sword. ROS serves as a signaling molecule to regulate many critical cellular processes including growth, proliferation, differentiation and apoptosis and also promotes oxidative stress and stimulates pathogenic conditions at increased levels [2,37]. Although under a physiological state, a dynamic equilibrium is produced by regulating different signaling pathways, antioxidant agents are required to trigger the homeostasis of ROS in a pathogenic

state. It is well known, that CeO_2-NPs affect antioxidant pathways via regulation of ROS levels or direct interactions with proteins, as will be discussed in the following sections.

CeO_2-NP-targeted signaling pathways

Since the activation of signaling pathways is the major key to cellular responses and ultimately living system decisions, it is essential to consider them in order to predict the interactions between CeO_2-NPs and biological systems components. Previous studies have suggested that these nanoparticles are involved in modulating different signaling proteins (Table 1) and subsequently can induce a variety of cellular responses dependent or independent from free radical scavenging. For instance, Kong *et al.* in their research on the lifespan of photoreceptor cells showed the potential of CeO_2-NPs for up-regulation of some proteins such as TRX, NRF2, pERK, bFGF and FGFR, which promote cytoprotective processes and down-regulation of caspase-8 and BAK 1 and the activation of caspases-9 and -3 that are associated with apoptosis and discussed the relevant signaling pathways [38]. In another study, Cai *et al.* confirmed the effects of CeO_2-NPs on the expression of antioxidant genes [39]. In the following sections, a detailed explanation of the most common CeO_2-NP-targeted proteins is provided with a description of how these factors relate to antioxidant activity.

NRF2

There seems to be an overall agreement that NRF2 as a transcription factor plays a key role in antioxidant defense system. ROS-activated NRF2 in the nucleus binds antioxidant-responsive elements and attenuates elevated ROS levels. Under basal conditions this molecule is targeted for proteasomal degradation through binding to KEAP1 [2,40]. Thus, NRF2 could be a target for therapeutic approaches in the clinic as today a variety of drugs are designed for its regulation under special conditions. Interestingly, in some literatures it has been reported that CeO_2-NPs regulate the expression of NRF2-associated genes. For example, Hasanvand *et al.* investigated *Nrf2* expression and *Ho-1*, *Nqo1* and *Gclc*, which are its downstream antioxidant genes in streptozotocin (STZ)-induced diabetic rats after exposure to CeO_2-NPs. Their results confirmed that CeO_2-NPs reduce oxidative stress damages through up-regulating these cytoprotective genes [41]. Enhancement of *Nrf2* and its associated genes which lead to alleviating damages related to oxidative stress-induced diseases is reported in inherited early progressive cochlear and retinal degeneration tubby mice [38], STZ-induced diabetic mice [42], primary astrocytes in $Nrf2^{-/-}$ mice [43] and HepG2 cells [44]. Conversely, some studies have shown reduction of *Ho-1* and *Nrf2* in D-GALN/lipopolysaccharide (LPS)-induced hepatotoxicity [45] and down-regulation of *HO-1* and *SOD2* genes in the $KBrO_3$-treated BEAS-2B cells (a human epithelial lung cell line as a pulmonary-like cell system) [46] where intracellular ROS was quenched. Likewise, others have exhibited CeO_2-NP-induced oxidative stress, inflammation and DNA damage via overexpression of *Nrf2*, *Sod* and *Ho-1* genes in a rat model of cisplatin-induced vascular injury [47] and human bronchial epithelial cells [48].

NF-κB

NF-κB is a transcription factor that participates in regulation of various physiological processes, including inflammation and immune response [49]. This protein is normally inhibited via binding IκB family in the cytoplasm. The phosphorylation and subsequently ubiquitination of inhibitor proteins lead to activation and translocation of NF-κB into the nucleus that promotes expression of target genes in response to microbial products, stress and pro-inflammatory cytokines. The mechanisms involved in activation of NF-κB pathway are canonical and non-canonical or alternative pathways that rely on inhibition of IκB and processing of NF-κB2 precursor protein and P100, respectively [50]. In addition, it has been clarified that NF-κB contributes to pathophysiological conditions such as cancer, so many studies have investigated modulators of this pathway and therapeutic approaches associated with them. For instance, it has been reported that CeO_2-NPs decrease ROS levels and suppress phosphorylation of IκBα and the translocation of P65 subunits of NF-κB into the nuclei in H9c2 cardiomyocytes exposed to cigarette smoke extract [51]. Moreover, these NPs reduce *Nf-κb* expression and subsequently alleviate side effects of inflammatory and immune response in the STZ-induced diabetic mice [42] as well as the NRF2-deficient mice [43]. Furthermore, diminution of oxidative stress and increment of up-regulated NF-κB genes have been indicated in rats with drug-induced hepatotoxicity [52] and in very low-density lipoprotein receptor knockout ($Vldl^{-/-}$) mice [39]. Given the fact that previous findings demonstrated ROS activity in NF-κB signaling was dependent on the activated intracellular pathway and the cell type, it is reasonable that CeO_2-NP can function as modulator of NF-κB signaling selectively through regulation of ROS in canonical pathway.

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Table 1. Effects of cerium oxide nanoparticles on different signaling pathways.

Morphology	Size (nm)	HR, nm	Con. and delivery route	Study type	Results	Assay	Ref.
NR	10	20	7.0 mg/kg	*In vivo*, rat lungs	Activation of MAPK	Immunoblotting analysis	[56]
NR	NR	NR	0.15, 0.5, 1, 3.5 and 7 mg/kg, it.	*In vitro*, human bronchial epithelial cells	Elevated *P38* and *NRF2*	PCR and apoptosis assay (ELISA kit)	[16]
NR	NR	NR	0, 0.01, 0.1, 0.5, 1 and 10 μg/ml	*In vitro*, BGC823 and MKN28 gastric cancer cells	Expression of *DHX15* and activation of *P38 MAPK*, anticancer effects	Microarray, western blot analysis	[57]
Hexahedral	20–30	NR	12.5, 25, 50, 100 and 200 μg/ml	*In vitro*, human hepatoma SMMC-7721 cells	Phosphorylation of ERK1/2, JNK and P38 MAPK, anticancer effects	Western blot	[52]
NR	NR	NR	10 μM	*In vitro*, hTERT-HPNE normal cell and pancreatic cancer cell	Activation of TRX1, ASK1 and JNK MAPK CO-radiotherapy	Phospho-ELISA L3.6pl and western blot	[58]
NR	NR	NR	25, 50, 100, 200, 400 and 800 μg/ml	*In vitro*, Jurkat cell	Activation of P38 MAPK and NF-κB, anticancer effects	Western blot	[59]
NR	NR	NR	0.5 mg/ml, iv. 0, 1, 5, 10, 25, 50, 100 and 1000 ng/ml	*In vitro* and *in vivo* Raw 264.7 cells	Increased phosphorylation of P38-MAPK and P44/42-MAPK	Immunoblotting	[60]
NR	10	197	0, 1.5625, 3.125, 6.25, 12.5, 25, 50 or 100 μg/ml	*In vitro* HSF cell	Reduction of the expression of *p-JNKs*, *p-c-JUN* and *IL-6*, as well as the generation of IL-8 and MMP-2	Western blot, ELISA, quantitative RT-PCR	[62]
Spherical	3	40–45	iv.	*In vivo*	Promoting AMPK-PKC-CBP pathway and reducing caspase-9 and caspase-3	Western blot	[66]
NR	5–16	<200	10–100 μg/ml	*In vitro*, murine cell line C17.2 and human NPC	Nontoxic for neuronal cells but inhibited neurogenesis	NGS and RNA-Seq coupled with computational analysis of transcriptomics data	[67]
Cubic	5–80	230	10–100 μg/ml	*In vitro*, PC12 cells	Differentiation and dopamine secretion	Quantitative RT-PCR	[68]
NR	5–10	NR	1 mM = 172 ng/ml, iv.	*In vivo*, in $vldlr^{-/-}$ mice	Promoting ASK1-P38/JNK-NF-κB pathway and up-regulating *Nrf2*	Western blot, RT-PCR and PCR array	[63]
Different-simulation	5–10	NR	100 nm – 1 μM	*In vitro*, HUVEC cells	Induction of angiogenesis by regulating HIF-1α	Western blot and RT-PCR simulation	[69]
CeO_2-NPs (US, Research Nanomaterials, Inc)	NR	NR	30 mg/kg daily for 2 weeks	*In vivo*	Up-regulating *Ho-1*, *Gclc*, *NQO1* and *Nrf2*	Real-time RT-PCR	[41,70]
Cubic	8	323 in DMEM	10 μg/ml	*In vitro*, RAW 264.7 and BEAS-2B cells	CeO_2-NP was inert for *NRF2* expression	Real-time RT-PCR	[71]
Cerianite	8 and 58	NR	0.3, 3 and 30 μg/ml	*In vitro* HepG2 cells	Increasing *NRF2* expression by 58-nm particles	Microarray analysis	[44]
NR	NR	NR	5–200 ng/ml 0.5 mg/kg	*In vivo* and *in vitro* Primary astrocyte and $Nrf2^{-/-}$ mice	Enhancement of NRF2 and inhibition of astrocytes activation related NF-κB pathway	Real-time RT- PCR and western blot	[43]
NR	25	Administration	0.01 μg/kg	*In vivo*, D-GALN/LPS induced hepatotoxicity	Reduction of *Ho-1* and *Nrf2* in D-GALN/LPS induced hepatotoxicity	Real-time RT-PCR and western blot	[45]
NR	20	NR	1 mg/kg	*In vivo*, a rat model of CP-induced vascular injury	Enhancement of oxidative stress, *Nrf2* expression, inflammation and DNA damage	Immunohistochemical staining	[47]

CeO_2-NP: Cerium oxide nanoparticle; Con: Concentration; HR: Hydrodynamic radius; ip.: Intraperitoneal injection; it.: Intratracheal; iv.: Intravitreal; JNK: c-Jun amino terminal kinase; LPS: Lipopolysaccharide; NR: Not reported; ROS: Reactive oxygen species; SI: Systemic injection; SOD: Superoxide dismutase.

Table 1. Effects of cerium oxide nanoparticles on different signaling pathways (cont.).

Morphology	Size (nm)	HR, nm	Con. and delivery route	Study type	Results	Assay	Ref.
NR	90 ± 9.5	180 ± 15.54 intraperitoneally	0.2 and 2 mg/kg, ip. after streptozotocin	*In vivo*, streptozotocin-induced diabetic mice	Reduction of *Nf-κb* expression and enhancement of *Nrf2* expression	Immunohistochemistry	[42]
Spherical	4	37 nm in saline solution at pH = 5.5	0.1 mg/ml	*In vitro* and *in vivo* HepG2 and stress agents-exposed rats	Activation of NF-κB	Western blot and NF-κB transcription factor assay kit and ELISA	[72]
NR	NR	NR	1, 10 or 100 nM	*In vitro*, H9c2 cardiomyocytes exposed to cigarette smoke extract	Inhibiting of oxidative stress and NF-κB activation	NF-κB promoter assay and immunostaining	[51]
Cubic	9.52 ± 0.66	93.17 ± 5.10	2.5 μg/ml before addition of $KBrO_3$	*In vitro*, $KBrO_3$-treatment BEAS-2B cells	Down-regulation of *HO1* and *SOD2*, quench of ROS	Real-time RT-PCR	[46]
NR	15–45	76–1588	1 mg/l	*In vitro*, human bronchial epithelial cells	Induction of P38, NRF2, SOD, HO-1 as well as ROS and inert for NF-κB	Western blot	[48]
NR	50		0–200 μg/ml	*In vitro*, A549 cells	Enhancement of *TGF-β* expression	Real-time RT-PCR	[73]
NR	3–5	NR	172 ng/μl (1 mM), SI	*In vivo*, tubby mice	Up-regulation of TRX, NRF-2, pERK, bFGF and FGFR	Western blot	[38]
NR	3–5	NR	172 ng/μl (1 mM)	*In vivo*, tubby mice	Up-regulation of antioxidant genes	qRT-PCR and western blot	[39]
NR	NR	As nanoliposomes	Different	*In vitro*, human prostate cancer cells	Inhibition of PI3K-AKT pathway	Real-time RT-PCR and western blot	[65]
Spherical	5–9	110.7 and 283.3	0.1, 0.5, 10, 100 μg/kg, IP	*In vivo*, hypobaric hypoxia rat	Inflammatory factors protective effect on lung	ELISA	[18]

CeO_2-NP: Cerium oxide nanoparticle; Con: Concentration; HR: Hydrodynamic radius; ip.: Intraperitoneal injection; it.: Intratracheal; iv.: Intravitreal; JNK: c-Jun amino terminal kinase; LPS: Lipopolysaccharide; NR: Not reported; ROS: Reactive oxygen species; SI: Systemic injection; SOD: Superoxide dismutase.

MAPKs

The MAPKs are involved in different cellular processes including proliferation, differentiation, motility and apoptosis. Some examples of these molecules include ERK-1/2-MAPK (important in cell survival), P38 MAPK (as an apoptosis inducer), the c-Jun amino terminal kinase (JNK)-MAPK and the big MAP kinase 1 (BMK1/ERK5) [53,54]. MAPKs are induced by extracellular stimuli and regulate a wide range of cellular functions [55]. Recently, cumulative evidence has indicated that CeO_2-NPs influence the activation of the constituents of MAPKs cascade. Available information suggests that these nanoparticles also have the ability of both activating and inhibiting the MAPKs pathways in different environmental conditions. For instance, Rice *et al.* have indicated that CeO_2-NPs with approximately 10 nm physical diameter at a dosage of 7.0 mg/kg increased phosphorylation of P38 MAPK (activation) and decreased phosphorylation of ERK-1/2-MAPK (inhibition) after localization in rat lung which was associated with inflammation [56]. Another study on human bronchial epithelial cells also showed that CeO_2-NPs elevated phosphorylation of P38 and NRF2 [20]. It has been reported that CeO_2-NPs could also up-regulate the expression of putative ATP-dependent RNA helicase DEAH (Asp-Glu-Ala-His) box helicase 15 (DHX15) which is involved in spliceosomes and in turn stimulated P38 MAPK signaling pathway with an ROS-independent mechanism in gastric cancer cell lines [57]. Other studies have shown elevated phosphorylation levels of MAPK pathways (ERK1/2, JNK and P38) induced by CeO_2-NPs in human hepatoma SMMC-7721 cells [52] and increased cellular expression of *TRX1* which in turn could promote radiation therapy by inducing JNK activation in L3.6pl human pancreatic cancer cells [58]. In addition, combination of ZnO and CeO_2-NPs had synergistic effects on P38 kinase activation in Jurkat cells [59]. Conversely, there are evidences that demonstrate CeO_2-NPs can function to block the activation of these signaling pathways. Reported results by Selvaraj *et al.* indicated an increased phosphorylation of P38-MAPK and P44/42-MAPK associated with exposure to LPS in septic rats that was attenuated after injecting CeO_2-NPs intravenously [60]. Another study indicated that CeO_2-NPs decreased P38, ERK-1/2 and SAPK/JNK phosphorylation and activation in cultured RAW 264.7 macrophage cells in the presence of LPS [61] and therefore

led to enhanced animal and cell survival against LPS-induced sepsis. Interestingly, UVA photoprotective potential of CeO_2-NPs was demonstrated which resulted in overexpression of phosphorylated JNK pathway in human skin fibroblasts that overcame oxidative stress and led to prevention of skin photoaging [62]. Besides, in order to evaluate how CeO_2-NPs exert their therapeutic effects such as prevention of pathologic vascular leakage in mice with age-related macular degeneration, Cai *et al.* examined regulation of ASK1-P38/JNK-NF-κB signaling pathway. They showed that CeO_2-NPs significantly increased the expression of ASK1, P38 and JNK as well as NF-κB [63].

Since MAPKs signaling pathways induced by CeO_2-NPs have been observed in cancer cells and also *in vivo* in studies related to lung with acidic pH (6.69 ± 0.07) [64] and according to other cases pointed above (physiological pH), the differences in CeO_2-NPs behavior presumably can be explained by altering the environmental pH.

The PI3K-AKT pathway

The PI3K-AKT pathway triggers a series of cellular processes such as protein synthesis, cell cycle progression and proliferation and suppresses some others including apoptosis, autophagy and drug resistance. Activated PI3K proteins in response to various stimuli promote catalyzing the synthesis of phosphatidylinositol 3,4,5-triphosphate (PIP3). Afterward, the PDK and PKB (also named as AKT) are activated and affect on involved proteins in cellular functions through phosphorylation. In addition, PTEN is one of the major factors in PI3K-AKT signaling pathway which negatively regulates it. Therefore, identifying effective drugs on this pathway is important, since a breakdown in each component could be a threat for cellular function and can lead to a variety of disorders. In line with these observations, Singh *et al.* designed CeO_2-NP and a plasmid expressing PTEN-encapsulated nanoliposomes (containing different concentrations of nanoparticles) to suppress growth and viability of PC-3, a human prostate cancer cell line. This study indicated that these antioxidant nanoliposomes induced apoptosis in cancer cells through increasing PTEN and decreasing AKT-1, AKT-2, AKT-3 and mTOR which resulted in increased expression of caspase-7 mRNA. Their results indicated that treatment potential of this unique class of nanoliposomal anticancer agent depends on concentration of nanoparticles and encapsulation efficiency to control CeO_2-NPs behaviors in biological systems [65].

Conclusion

For understanding and subsequently predicting the behavior of cells or organisms in response to foreign invaders or drugs it is important to have an overview of the complex communications of their component molecules or in other words signal transductions.

In the past two decades, CeO_2-NPs have been discussed as both oxidant and antioxidant agents. CeO_2-NPs have achieved considerable research interests in biomedical applications because of their selective manner abilities in different microenvironments such as cancer or oxidative stress-induced diseases versus physiological state. Different factors have been considered and discussed to explain these inconsistent behaviors of CeO_2-NPs. These factors include specific properties of nanoparticles, for example size, shape, surface charge, concentration, instillation (inhalation, intratracheal etc.), synthesis and dispersion methods on one hand and components of biological systems on the other hand. Thus, cellular decisions depend on connections of a wide network of molecular mechanisms and signaling pathways in a dynamic state. Since the effects of CeO_2-NPs are mainly related to ROS modulation, we discussed some of the CeO_2-NP-targeted signaling pathways which are related to ROS including NRF2, MAPK, NF-κB and PI3K pathways. As explained above, effects of CeO_2-NPs on cellular signaling pathways can result in different biological responses and this complication could be due to biological system dynamic behavior arising from the diversity of physical and physiological factors related to both CeO_2-NPs and biological systems.

Taken together, in order to use the capacities of CeO_2-NPs for diagnostic, treatment and even personalized medicine applications, because of dynamic state of biological systems, it is important to have adequate knowledge and fully understand the CeO_2-NP-targeted signaling pathways in various complex cellular environments. Use of this information in a systems biology approach would be very beneficial in predicting cellular behavior and would improve application of these NPs for clinical purposes.

Future perspective

In a biological system, cell behavior is determined by collaborative interactions of its component molecules. Being controlled by a complex network of molecular mechanisms and a dynamic state in biological systems have made it difficult to predict the exact cellular response to various signals including nanoparticles. In recent years, advances in systems biology and novel simulation approach have provided an unprecedented opportunity to have a better

understanding of cellular activities in different conditions. It is important to emphasize that this approach can only be effective and promising when it is integrated and aligned with experimental data. Thus, integrating the effects of CeO_2-NPs on different molecular pathways with systems biology would be the way forward in future studies on this nanoparticle.

Executive summary

Cerium oxide nanoparticle (CeO_2-NP): a flexible nanoparticle

- CeO_2-NPs can act as both pro- and anti-oxidant agents depending on their characteristics and environmental conditions.
- CeO_2-NPs can be used for drug delivery, tissue engineering, radiation protection and treatment of diseases related to reactive oxygen species such as cancers, neurological disorders, autoimmune degenerative diseases, cardiovascular diseases – among others.
- Inconsistent potential of CeO_2-NPs is associated with switching between Ce^{3+} and Ce^{4+} states.

Signaling pathways

- Signaling pathways play key roles in critical cellular functions including growth, proliferation, differentiation, division and apoptosis.

CeO_2-NP-targeted signaling pathways

- CeO_2-NP as a ligand can interact with many biomolecules triggering a cascade of cellular activities.
- CeO_2-NP-targeted signaling pathways can induce a variety of cellular responses dependent or independent from free radical scavenging.
- Some free radical scavenging-dependent proteins include NRF2, MAPK, NF-κB and PI3K.
- New and effective strategies such as computational modeling and systems biology can be used to understand this neglected concept.

Financial & competing interests disclosure

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•• **The authors explore the efficiency of cerium oxide nanoparticles (CeO_2-NPs) in treatment of different types of cancers and point out their potentials as invasion inhibitors, radioprotection and radio-sensitization agents, as well as their effects in controlling angiogenesis.**

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