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Involvement of Programmed Cell Death in Neurotoxicity of Metallic Nanoparticles: Recent Advances and Future Perspectives

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Abstract

The widespread application of metallic nanoparticles (NPs) or NP-based products has increased the risk of exposure to NPs in humans. The brain is an important organ that is more susceptible to exogenous stimuli. Moreover, any impairment to the brain is irreversible. Recently, several in vivo studies have found that metallic NPs can be absorbed into the animal body and then translocated into the brain, mainly through the blood–brain barrier and olfactory pathway after systemic administration. Furthermore, metallic NPs can cross the placental barrier to accumulate in the fetal brain, causing developmental neurotoxicity on exposure during pregnancy. Therefore, metallic NPs become a big threat to the brain. However, the mechanisms underlying the neurotoxicity of metallic NPs remain unclear. Programmed cell death (PCD), which is different from necrosis, is defined as active cell death and is regulated by certain genes. PCD can be mainly classified into apoptosis, autophagy, necroptosis, and pyroptosis. It is involved in brain development, neurodegenerative disorders, psychiatric disorders, and brain injury. Given the pivotal role of PCD in neurological functions, we reviewed relevant articles and tried to summarize the recent advances and future perspectives of PCD involvement in the neurotoxicity of metallic NPs, with the purpose of comprehensively understanding the neurotoxic mechanisms of NPs.

Keywords: Brain, Apoptosis, Autophagy, Necroptosis, Pyroptosis, Nanoparticles

Review

Introduction

With the rapid development of nanotechnology, metallic (metal or metal oxide) nanoparticles (NPs), with a diameter ranging from 1 to 100 nm, are used in cosmetics [1], food addictives [2], building industry [3], paints [4], battery [5], and biomedical applications [6], owing to their extraordinary physicochemical properties. Metallic NP-based products facilitate our daily life; however, it has several disadvantages. The widespread application of NP-based products increases the risk of exposure to metallic NPs for humans, especially for those who work in industries involving the use of these materials. [7]. In addition, several in vivo studies have demonstrated that once animals are exposed to metallic NPs through intravenous injection [8], intranasal instillation [9], oral administration [10], inhalation [11], and intraperitoneal injection [12], NPs can be absorbed into the body and then re-distributed into secondary organs, such as the brain, liver, spleen, lungs, and kidneys. The brain is an important organ susceptible to harmful substances [13–15], and impairment to the brain is irreversible. Accumulated metallic NPs, an exogenous stimuli, induce apoptosis, up-regulate inflammatory responses, activate signaling pathways, disturb the neurotransmitters, and impair organelles (such as mitochondria), and these changes contribute to the neurotoxicity of NPs, consequently leading to brain dysfunction. However, the mechanisms underlying the neurotoxicity of metallic NPs remain unclear.

Programmed cell death (PCD), which is different from necrosis, is defined as active cell death, which is regulated by certain genes. The role of PCD is to balance the proportion of dead cells and healthy cells and maintain homeostasis. In general, PCD can be classified into apoptosis, autophagy, necroptosis, and pyroptosis [16, 17]. PCD can either be observed under physiological conditions or be

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induced by exogenous stimuli. Moreover, PCD is reported to be closely related to brain development [18, 19], neurodegenerative disorders [20, 21], brain injury [22, 23], and psychiatric disorders [24]. Based on the important role of PCD in neurological functions, in this review, we summarized the recent advances and put forward some suggestions regarding the involvement of PCD in the neurotoxicity of metallic NPs by analyzing relevant articles. We expect that investigating the correlation between PCD and metallic NPs can help us to understand the mechanisms underlying the neurotoxicity of NPs completely.

Accumulation of Metallic NPs in the Brain After Systemic Administration

The brain is the main target of metallic NPs, and brain damage is irreversible. Therefore, more attention should be paid to the threat posed by metallic NPs on brain health. Metallic NPs can be absorbed into the body and then translocated into the brain, mainly through the blood–brain barrier (BBB) and direct nose-to-brain (or olfactory) pathway bypassing the BBB.

The results of several in vivo studies have revealed that metallic NPs can be detected in animal brain after systemic administration. Rats showed higher anxious index, which indicated impaired neurobehavioral functions, and the contents of TiO_2 NPs in the brain, lungs, and liver were elevated, owing to TiO_2 NP exposure via intraperitoneal injection every 2 days for 20 days [12]. The concentrations of TiO_2 NPs in the brain, liver, spleen, kidneys, lungs, and heart were elevated after the rats were exposed to NPs through single or repeated intravenous injection [8]. When the rats were exposed to silver NPs through chronic intranasal instillation, the brain subunits including the cortex, hippocampus, cerebellum, olfactory bulb, and medulla exhibited higher contents of NPs [9]. The exposure to silver NPs through single intravenous injection can also increase the NP levels in the mouse brain [25, 26]. Meanwhile, an oral administration was able to increase the content of silver NPs in the rat brains as well [10, 27–29].

In addition to TiO_2 and silver NPs, exposure to gold NPs through single intravenous injection increased the concentration of NPs in the mouse brain [30]. Inhalation administration for 15 days led to elevated gold NP levels in the subunits of the brain including the olfactory bulb, hippocampus, striatum, frontal cortex, entorhinal cortex, septum, and cerebellum [11]. The gold NP content in the rat brain was enhanced 24 h after the intravenous injection [31]. Zinc oxide (ZnO) NP exposure through repeated oral administration slightly increased the ZnO NP levels in the rat brains [32]. The content of copper (Cu) NPs in the olfactory bulb increased after the mice were chronically exposed to Cu NPs through intranasal instillation [33, 34].

Even worse, the metallic NPs can cross the placental barrier and accumulate in fetal organs on exposure to NPs during pregnancy. When pregnant mice were intravenously injected with silica and TiO_2 NPs, both NPs were detected in the fetal brain, fetal liver, and placenta 24 h after the injection. These changes might impair the fetal development including neurodevelopment, which indicates that developmental neurotoxicity can be induced by silica and TiO_2 NPs [35]. We must pay much attention to the neurodevelopmental toxicity of metallic NPs, as fetal brain is more susceptible to harmful stimuli.

To sum up, investigating the bio-distribution of metallic NPs might help us to screen the safest metallic nanomaterials and administration routes that can protect the brain from being affected by NPs. Therefore, relevant studies should be further performed in the future. In addition, studies should be conducted to comprehensively investigate the relationship between exposure to metallic NPs during pregnancy and fetal brain development.

The Contribution of PCD to the Neurotoxicity of Metallic NPs

As mentioned above, metallic NPs can be translocated into the brain after systemic administration. This accumulation in turn can lead to neurotoxicity. PCD as an active cell death process mainly consists of apoptosis, autophagy, necroptosis, and pyroptosis. Moreover, PCD plays an important role in neurological functions. Therefore, we will discuss the correlation between PCD and the neurotoxicity of metallic NPs, with the purpose of comprehensively understanding the neurotoxic mechanisms of NPs.

Apoptosis—Established Role in the Neurotoxicity of Metallic NPs

Apoptosis is the first and most commonly studied PCD type. It can be simply defined as programmed "self-killing" [36]. Apoptosis plays an important role in cell renovation and elimination of injured cells. Dysregulation of cell apoptosis can induce cell death and impairment of tissues, consequently leading to organ dysfunction [37]. Human health and diseases can be regulated by cell apoptosis [37, 38]. Apoptosis is mediated by caspase-dependent pathways (Fig. 1) [39, 40]. Generally, apoptosis is characterized by blebbing, DNA fragmentation, and caspase activation [41–43].

In Vitro Studies Related to Apoptosis in Neurotoxicity of Metallic NPs Long et al. first reported that TiO_2 NPs can induce apoptosis in immortalized mouse microglia (BV2), rat dopaminergic neuronal cells (N27), and primary embryonic rat stratum neurons [44]. Another research group revealed that the proportion of

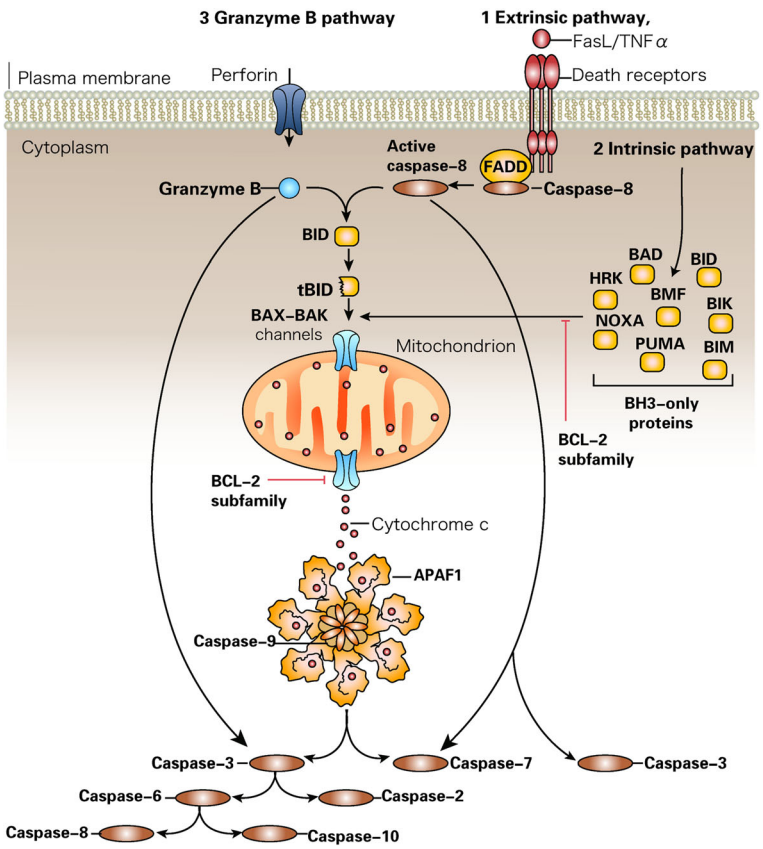

Fig. 1 Caspase activation pathways [131]. Caspase activation by the extrinsic pathway (route 1) involves the binding of extracellular death ligands (such as FasL or tumor necrosis factor-α (TNFα)) to transmembrane death receptors. Engagement of death receptors with their cognate ligands provokes the recruitment of adaptor proteins, such as the Fas-associated death domain protein (FADD), which in turn recruit and aggregate several molecules of caspase-8, thereby promoting its autoprocessing and activation. Active caspase-8 then proteolytically processes and activates caspase-3 and caspase-7, provoking further caspase activation events that culminate in substrate proteolysis and cell death. In some situations, extrinsic death signals can crosstalk with the intrinsic pathway through caspase-8-mediated proteolysis of the BH3-only protein BID (BH3-interacting domain death agonist). Truncated BID (tBID) can promote mitochondrial cytochrome *c* release and assembly of the apoptosome (comprising ~7 molecules of apoptotic protease-activating factor-1 (APAF1) and the same number of caspase-9 homodimers). In the intrinsic pathway (route 2), diverse stimuli that provoke cell stress or damage typically activate one or more members of the BH3-only protein family. BH3-only proteins act as pathway-specific sensors for various stimuli and are regulated in distinct ways. BH3-only protein activation above a crucial threshold overcomes the inhibitory effect of the anti-apoptotic B cell lymphoma-2 (BCL-2) family members and promotes the assembly of BAK–BAX oligomers within mitochondrial outer membranes. These oligomers permit the efflux of intermembrane space proteins, such as cytochrome *c*, into the cytosol. On release from mitochondria, cytochrome *c* can seed apoptosome assembly. Active caspase-9 then propagates a proteolytic cascade of further caspase activation events. The granzyme B-dependent route to caspase activation (route 3) involves the delivery of this protease into the target cell through specialized granules that are released from cytotoxic T lymphocytes (CTL) or natural killer (NK) cells. CTL and NK granules contain numerous granzymes as well as a poreforming protein, perforin, which oligomerizes in the membranes of target cells to permit entry of the granzymes. Granzyme B, similar to the caspases, also cleaves its substrates after Asp residues and can process BID as well as caspase-3 and caspase-7 to initiate apoptosis. *BAD* BCL-2 antagonist of cell death, *BAK* BCL-2-antagonist/killer-1, *BAX* BCL-2-associated X protein, *BID* BH3-interacting domain death agonist, *BIK* BCL-2-interacting killer, *BIM* BCL-2-like-11, *BMF* BCL-2 modifying factor, *HRK* harakiri (also known as death protein-5), *PUMA* BCL-2 binding component-3

apoptotic cells in human astrocyte-like cell lines (U87) increased on TiO_2 NP exposure [45]. TiO_2 NP exposure inhibited cell proliferation, increased the proportion of apoptotic cells, activated caspase-3 in rat (C6) and human (U373) glial cell lines, and was accompanied by hyper-condensed nuclei [46]. TiO_2 NPs can also attenuate cell viability and increase the number of apoptotic cells after the exposure of murine microglia cell lines (N9) to NPs [47]. Besides, the mitochondrial membrane potential (MMP) of PC12 cells was reduced by TiO_2 NP exposure, whereas the proportion of apoptotic cells was enhanced. The expression of Bax and p53 was up-regulated, while that of Bcl-2 was down-regulated at the protein level. Meanwhile, TiO_2 NP exposure promoted the activity of caspase-3 [48]. These changes indicated that apoptosis in PC12 cells was induced by TiO_2 NPs.

Cell viability was not reduced by TiO_2 NPs; however, the cell cycle was disturbed on exposure to TiO_2 NPs. An increased proportion of apoptotic cells and up-regulated MMP were observed in human neuronal cell lines (SH-SY5Y) [49].

After the rat primary cortical neurons were exposed to silver NP, cell viability decreased, the protein levels of caspase-3 increased, the proportion of apoptotic cells increased, and a DNA ladder was observed. These findings suggested that silver NP induced apoptosis, which led to neurotoxicity [50]. Silver NP can reduce cell viability, increase the number of apoptotic cells, enhance reactive oxygen species (ROS) production, and activate caspase-3 in rat primary neurons. In vivo experiments further confirmed that silver NP can be detected in the rat brain, and after intranasal administration, they can in turn up-regulate the protein levels of caspase-3, which was consistent with the results observed in the in vitro experiment. These findings suggested that silver NP exposure can induce caspase-dependent apoptosis contributing to neurotoxicity [51]. Another study revealed that silver NPs reduced cell viability and increased the number of apoptotic cells in PC12, which further verified the role of caspases in apoptosis. Co-treating PC12 cells with caspase-8 or caspase-9 inhibitors attenuated the apoptosis induced by NPs, which suggested that both death receptor-regulated signaling and mitochondrial-mediated pathway are involved in silver NP-induced apoptosis in PC12 cells [52]. The proportion of apoptotic cells in rat primary astrocytes, determined by using DNA fragmentation assay [53] and flow cytometry [54], was elevated in the silver NP-treated group. The caspase activity was also enhanced in this group [54].

When the human neuroblastoma cell lines (SH-SY5Y) were treated with ZnO NPs, cell viability was reduced, swelling or loss of organelles was detected, the number of apoptotic cells increased, and the activities of caspase-3/7 were up-regulated [55]. A similar conclusion was reached by another study, which revealed that the number of apoptotic cells in SH-SY5Y was enhanced by ZnO NPs [56]. Mouse neural stem cells (NSCs) can be impaired by ZnO NPs. Cell viability was attenuated, accompanied by impaired morphology (such as membrane blebbing and hyper-condensed chromatin) and increased proportion of apoptotic cells, which indicated that apoptosis was induced by ZnO NPs [57]. After C6 cells were exposed to ZnO NPs, the NPs were taken up by the C6 cells, which resulted in the reduction of cell viability. Meanwhile, apoptotic-like morphological features such as blebbing, nucleus shrinkage, and hyper-condensed chromatin were observed. The number of apoptotic cells increased as well [58]. ZnO NPs induced apoptosis in U87 cells, which was characterized by condensed chromatin, nuclear fragmentation, and the increase in the apoptotic cell proportion [59].

The neurotoxicity induced by TiO_2, silver, and ZnO NPs was widely investigated. In addition, other metallic nanomaterials were able to induce apoptosis in neuronal cells.

Viability of PC12 was reduced, and the number of apoptotic cells was elevated by copper NPs [60]. Iron oxide NPs can also decrease the cell viability and induce apoptosis in PC12 cells, characterized by the increased proportion of apoptotic cells and up-regulated protein levels of p53 and Bax, as well as down-regulated Bcl-2 protein expression [61].

In Vivo Studies Related to Apoptosis in the Neurotoxicity of Metallic NPs TiO_2 NPs can be detected in the mouse hippocampus after intragastric treatment for 60 days. The accumulated TiO_2 NPs can in turn induce apoptotic-like changes in cell morphology (such as condensed chromatin and shrinkage of the nuclear membrane) and DNA ladder. Meanwhile, the expression of caspase-3, caspase-9, Bax, and cytochrome c was up-regulated accompanied by the down-regulated expression of Bcl-2 at gene and protein levels. These findings suggested that an intrinsic apoptosis pathway in the mouse hippocampus was induced by TiO_2 NP exposure, which resulted in impaired spatial recognition ability [62]. TiO_2 NPs can be detected in the rat brain after intravenous injection, once a week for 4 weeks. This accumulation increased the number of apoptotic cells, induced DNA ladder, activated caspase-3, up-regulated the expressions of p53, Bax, and cytochrome c, and down-regulated Bcl-2 expression at gene and protein levels. These results indicated that mitochondria-mediated apoptosis in the rat brain was induced by TiO_2 NPs [63]. After the mice were exposed to TiO_2 NPs for 90 consecutive days through intranasal instillation, NPs were detected in the mouse brain. Meanwhile, the proportion of apoptotic cells in the hippocampus increased, as indicated by apoptotic morphology (shrinkage of the nucleus, condensed chromatin, and swollen mitochondria), and the expression of genes related to apoptosis, determined by DNA microarray analysis, was altered [64]. Exposure to TiO_2 NPs during pregnancy via subcutaneous injection can alter the expression of genes related to apoptosis, which were determined by DNA microarray analysis in the brain of mouse offsprings [65]. These changes indicated that TiO_2 NP exposure could induce developmental neurotoxicity.

Silver NPs can increase the number of apoptotic cells in the rat hippocampal subunits (CA1, CA2, CA3, and DG) after oral administration for 28 days [66]. Exposure to silver NPs during pregnancy can also elevate the proportion of apoptotic cells in the hippocampal subunits of rat

offsprings [67]. Meanwhile, silver NPs can be taken up by human embryonic neural precursor cells (HNPCs), thereby inducing apoptosis in HNPCs [68]. These findings indicated that apoptosis was also probably implicated in the developmental neurotoxicity of silver NPs.

Rats exposed to CuO NPs through intraperitoneal injection once a day for 14 days performed poorly in the Morris water maze (MWM) test and long-term potentiation (LTP) was affected, which indicated that the rat hippocampus was impaired by NPs. At the same time, both the activity of caspase-3 and the levels of 4-hydroxynonenal (HNE) in the rat hippocampus were up-regulated in the NP-treated group when compared to that in the control group, indicating an apoptotic process in the hippocampal zone [69]. Apoptosis induced by gold NPs also resulted in neurotoxicity. A study involving in vivo and in vitro experiments demonstrated that gold NP exposure activated caspase-3 and increased the number of apoptotic SH-SY5Y cells. The in vivo experiment showed that gold NPs can be detected in the mouse brain and can promote caspase-3 activity after intravenous injection, which was consistent with the results of the in vitro experiment [70]. Aluminum oxide NP exposure through intranasal instillation damaged the mouse neurobehavioral function, accompanied by reduced MMP, increased apoptotic cells, and up-regulated caspase-3 gene expression [71] in the mouse brain.

Regulation of Neuronal Apoptosis Induced by Metallic NPs Although apoptosis was involved in the neurotoxicity of metallic NPs, the molecular mechanisms by which the NPs regulated apoptosis are unknown. Studies showed that oxidative stress (OS) status was related to cell apoptosis [72–74]. Therefore, a few rescue studies were conducted to verify the role of NP-induced OS in nanoneurotoxicity. TiO_2 NP exposure decreased cell viability and increased ROS production in PC12 cells; it also increased the proportion of apoptotic PC12 cells. However, pretreating PC12 cells with N-acetylcysteine (NAC) can reverse these changes. These findings suggested that the apoptosis in PC12 was probably mediated by TiO_2 NP-induced ROS [75], as NAC had an antioxidant property [76, 77]. Treatment with ZnO NPs can lead to decreased cell viability, excessive ROS production, and apoptotic morphology, such as nuclear shrinkage in primary astrocytes. Meanwhile, the reduction in MMP suggested that the intrinsic apoptotic pathway was implicated in neurotoxicity. Further experiments found that the proportion of apoptotic astrocytes increased. At the same time, the expression of Bax, cleaved poly-ADP-ribose polymerase (PARP), and cleaved caspase-3 was up-regulated at the protein level. However, the level of Bcl-2 protein decreased on treatment with ZnO NPs. Pretreatment of astrocytes with NAC or Jun N-terminal kinase (JNK) inhibitor could reverse the harmful effects induced by metallic NPs, which indicated that apoptosis was probably caused by NP-induced ROS through the JNK pathway [78]. ZnO NPs can reduce PC12 viability and increase the number of apoptotic PC12 cells, which was determined using flow cytometry. However, pretreating PC12 cells with N-(mercaptopropionyl)-glycine (N-MPG) can lead to the inhibition of apoptotic process, which indicated that apoptosis in PC12 might be mediated by ZnO NP-induced OS [79]. N-MPG is another type of ROS scavenger [80]. Copper oxide (CuO) NPs can decrease the mouse hippocampal cell line (HT22) viability and increase the number of apoptotic HT22; they also up-regulated Bax gene levels and down-regulated Bcl-2 mRNA levels in HT22. Meanwhile, the OS status in HT22 cells was disrupted. However, pretreating HT22 cells with crocetin can attenuate those harmful impacts. These findings indicated that CuO NP-induced apoptosis in HT22 cells was probably mediated by NP-induced OS [81]. Crocetin possessed antioxidant and neuroprotective capabilities and could counteract OS [82–84]. These results indicated that apoptosis was most probably initiated by metallic NP-induced OS. However, more rescue studies are needed to further confirm it. In addition to OS mechanism, other potential mechanisms should be investigated.

The findings from the above-mentioned in vitro and in vivo studies demonstrated that metallic NP-induced apoptosis was involved in the neurotoxicity of NPs. Meanwhile, a few rescue studies revealed that apoptosis in neurotoxicity was probably regulated by metallic NP-induced OS. In addition, findings were mostly obtained from in vitro studies. Furthermore, except TiO_2 NPs, ZnO NPs, and silver NPs, other metallic nanomaterials were less studied. Besides, metallic NPs can cross the placental barrier to affect fetal brain development, but studies about the involvement of apoptosis in developmental neurotoxicity of metallic NPs were scarce.

Autophagy—Role in the Neurotoxicity of Metallic NPs Needs Further Verification

Recently, autophagy has become a hot topic and has attracted much attention. It can be simply defined as programmed "self-eating" [36]. Autophagy is different from apoptosis, and is mediated by caspase-independent pathways. It can be identified as a particular accommodation of cells to starvation. The process of autophagy includes cell degradation, in which the cargo in the cytoplasm is transported into the lysosome. Autophagy is a dynamic recycling system and it can maintain cellular renovation and homeostasis [85]. It can be classified into microautophagy, macroautophagy, and chaperone-mediated autophagy (CMA) (Fig. 2) [86, 87].

Many studies revealed that metallic NPs can induce autophagy in non-neuronal cells including human

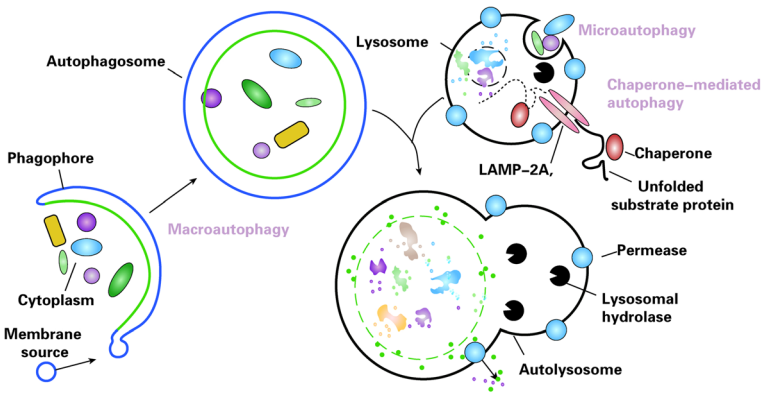

Fig. 2 Different types of autophagy [132]. Microautophagy refers to the sequestration of cytosolic components directly by lysosomes through invaginations in their limiting membrane. The function of this process in higher eukaryotes is not known, whereas microautophagy-like processes in fungi are involved in selective organelle degradation. In the case of macroautophagy, the cargoes are sequestered within a unique double-membrane cytosolic vesicle, an autophagosome. Sequestration can be either nonspecific, involving the engulfment of bulk cytoplasm, or selective, targeting specific cargoes such as organelles or invasive microbes. The autophagosome is formed by expansion of the phagophore, but the origin of the membrane is unknown. Fusion of the autophagosome with an endosome (not shown) or a lysosome provides hydrolases. Lysis of the autophagosome inner membrane and breakdown of the contents occurs in the autolysosome, and the resulting macromolecules are released back into the cytosol through membrane permeases. Chaperone-mediated autophagy (CMA) involves direct translocation of unfolded substrate proteins across the lysosome membrane through the action of a cytosolic and lysosomal chaperone hsc70, and the integral membrane receptor lysosome-associated membrane protein type 2A (LAMP-2A)

keratinocytes (HaCaT) [88], normal lung cells [89], MRC-5 fibroblasts [90], immune cells [91], human hepatocellular carcinoma HepG2 cells [92], murine peritoneal macrophage cells (RAW264.7) [93], and mouse embryonic fibroblasts [94]. Therefore, autophagy has been regarded as one of the mechanisms underlying nanotoxicity [95]. Moreover, it has been reported that autophagy was involved in neurotoxicity [96–98]. A few studies on the role of autophagy in the neurotoxicity of metallic NPs have been published. Kenzaoui et al. [99] found that the exposure of human cerebral endothelial cells (HCECs) to aminoPVA (poly(vinyl alcohol/vinylamine))-coated ultrasmall superparamagnetic iron oxide (USPIO) NPs, autophagic vacuoles were observed in HCECs and LC3-II. In addition, the cathepsin D protein levels were up-regulated, which suggested that autophagy in HCECs was induced by NPs. Manshian et al. [100] treated murine C17.2 neural progenitor cells with silver NPs and found that the LC3 fluorescent intensity was enhanced, which indicated that autophagy in C17.2 was induced by silver NPs. Since OS can induce autophagy [101–103], which was implicated in the neurotoxicity of metallic NPs [104–106], the role of autophagy in the neurotoxicity of metallic NPs should be not ignored.

Necroptosis and Pyroptosis—Potential Role in the Neurotoxicity of Metallic NPs

The role of necroptosis and pyroptosis in the toxicity of metallic NPs has not been extensively studied. Necroptosis, which can also be called "programmed necrosis," is initiated by activating the death receptor with stimuli (Fig. 3). Receptor-interacting protein kinases 1 and 3 are frequently involved in necroptosis [107, 108]. Studies on the relationship between necroptosis and nanotoxicity are rare. However, recent studies have demonstrated that cigarette can induce necroptosis in the mouse airway [109], carbon tetrachloride can lead to liver fibrosis via necroptosis [110], and glutamate can induce necroptosis in HT-22 cells [111].

Pyroptosis, a new type of PCD, is typically regulated by the caspase-1-dependent signaling pathway (Fig. 4). Caspase-1 is not involved in apoptosis or autophagy [112, 113]. It has been reported that silver NPs can induce pyroptosome formation in human monocytes (THP-1) and up-regulate the caspase-1 protein expression, which indicates that pyroptosis in THP-1 was induced by NPs [114].

The role of necroptosis and pyroptosis in the neurotoxicity of metallic NPs is uncertain. However, several reports have demonstrated that necroptosis and pyroptosis can be induced by OS [115–118], and metallic NP-induced OS contributed to neurotoxicity. Furthermore, necroptosis was involved in neurotoxicity induced by other harmful substances, such as iron [119], *Streptococcus pneumoniae* [120], and TNF-α [121]. Therefore, we hypothesized that metallic NP-induced OS can probably initiate necroptosis and pyroptosis, which might contribute to the neurotoxicity of NPs.

Future Perspectives

Based on the results of the above-mentioned studies, we put forward some suggestions for future research to

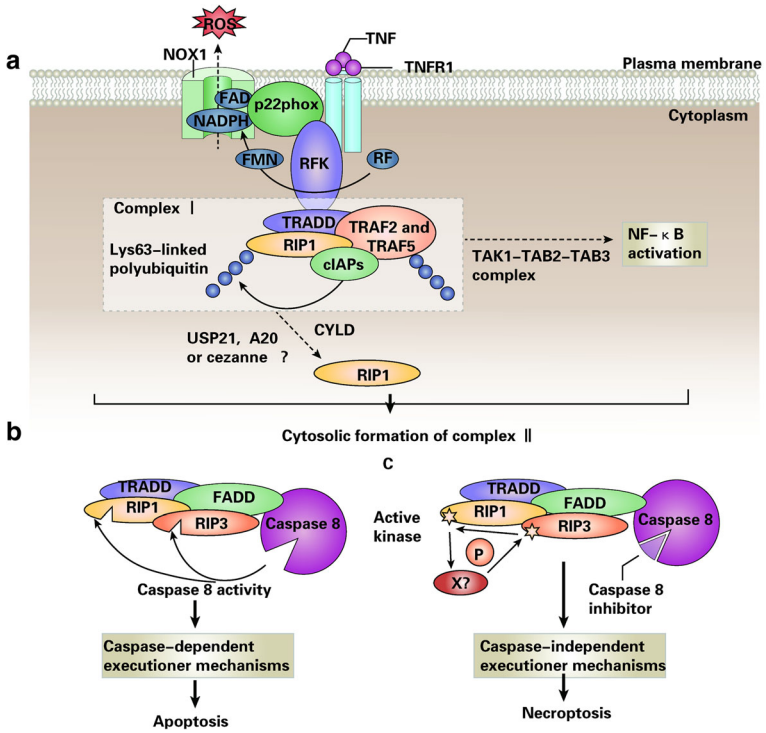

Fig. 3 TNFR1-elicited signaling pathways [133]. **a** On tumor necrosis factor (TNF) binding, TNF receptor 1 (TNFR1) undergoes a conformational change, allowing for the intracellular assembly of the so-called TNFR complex I, which includes TNF receptor-associated death domain (TRADD), receptor-interacting protein 1 (RIP1; also known as RIPK1), cellular inhibitor of apoptosis proteins (cIAPs), TNF receptor-associated factor 2 (TRAF2) and TRAF5. On cIAP-mediated Lys63-ubiquitylation, RIP1 can serve as a scaffold for the recruitment of transforming growth factor-β activated kinase 1 (TAK1) and TAK1-binding protein 2 (TAB2) and TAB3, which initiate the canonical nuclear factor-κB (NF-κB) activation pathway. Riboflavin kinase (RFK) physically bridges the TNFR1 death domain to p22phox (also known as CYBA), the common subunit of multiple NADPH oxidases, including NADPH oxidase 1 (NOX1), which also contributes to TNFα-induced necroptosis by generating reactive oxygen species (ROS). Conversely, on deubiquitylation by cylindromatosis (CYLD; and perhaps also by A20 (also known as TNFAIP3), cezanne (also known as OTUD7B) or ubiquitin-specific peptidase 21 (USP21)), RIP1 exerts lethal functions, which can be executed by two distinct types of cell death. **b** The internalization of TNFR1 is accompanied by a change in its binding partners that leads to the cytosolic assembly of TNFR complex II, which often (but not invariably) contains TRADD, FAS-associated protein with a death domain (FADD), caspase-8, RIP1, and RIP3 (also known as RIPK3). Normally, caspase-8 triggers apoptosis by activating the classical caspase cascade. It also cleaves, and hence inactivates, RIP1 and RIP3. **c** If caspase-8 is blocked by pharmacological or genetic interventions, RIP1 and RIP3 become phosphorylated (perhaps by an unidentified kinase) and engage the effector mechanisms of necroptosis. *FAD* flavin adenine nucleotide, *FMN* flavin mononucleotide

understand the role of PCD in the neurotoxicity of metallic NPs completely.

(1) Since the role of apoptosis in the neurotoxicity of metallic NPs has been widely studied, the signaling pathways through which NPs regulate neuronal apoptosis should be investigated comprehensively.
(2) As metallic NP exposure during pregnancy can affect fetal brain development [122–124], much attention should be paid to the role of apoptosis in developmental neurotoxicity induced by NPs.
(3) More in vivo studies are needed to further confirm the vital role of apoptosis in the neurotoxicity of metallic NPs.
(4) In addition to TiO_2 NPs and silver NPs that were most widely studied, other metallic nanomaterials including NPs of gold, copper, copper oxide, aluminum oxide, and iron oxide should be investigated to understand the pivotal role of apoptosis in the neurotoxicity of NPs completely.
(5) Several studies have already been performed to investigate the role of autophagy in non-neuronal cells [125–127], and autophagy was implicated in brain/neuron damage [128–130]. Although a few studies confirmed the involvement of autophagy in the neurotoxicity of metallic NPs, its role in neurotoxicity still needs further verification.
(6) Whether necroptosis or pyroptosis is involved in the neurotoxicity of metallic NPs should be investigated in the future.
(7) The correlation among apoptosis, autophagy, necroptosis, and pyroptosis in the neurotoxicity of metallic NPs should be studied.

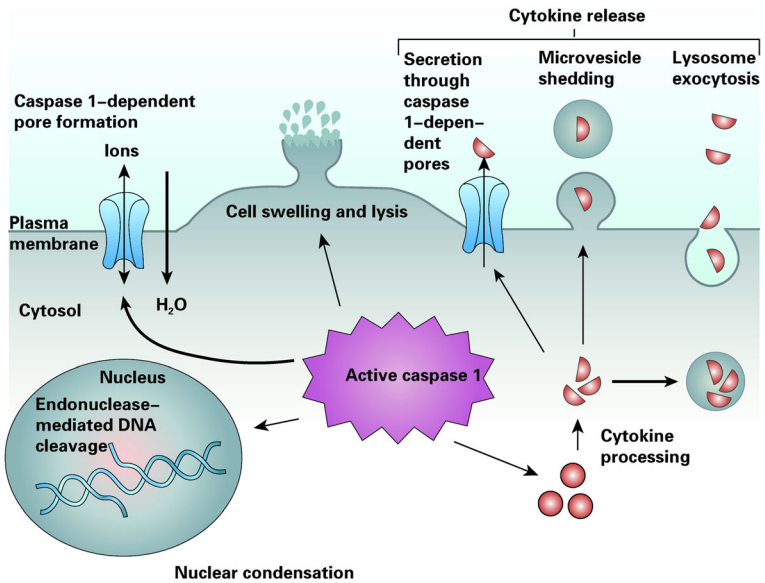

Fig. 4 Pyroptosis, an inflammatory host response [134]. Caspase-1 is cleaved and activated in response to multiple stimuli, but once activated, caspase-1 results in a conserved program of cell death referred to as pyroptosis. Caspase-1 activation also leads to rapid formation of plasma-membrane pores with a diameter of 1.1–2.4 nm. These pores dissipate cellular ionic gradients, allowing water influx, cell swelling, and osmotic lysis. The pro-forms of interleukin-1β (IL-1β) and IL-18 are processed by caspase-1 and released during pyroptosis, although the exact mechanism of secretion remains controversial. Secretion does not require lysis and is temporally associated with caspase-1-dependent pore formation, suggesting that these pores facilitate cytokine release. Other suggested secretion mechanisms include caspase-1-independent lysosome exocytosis and microvesicle shedding. Caspase-1 activity results in cleavage of chromosomal DNA by an unidentified endonuclease. Cleavage of DNA does not result in the oligonucleosomal fragments observed during apoptosis. Nuclear condensation is also observed but nuclear integrity is maintained, unlike the nuclear fragmentation observed during apoptosis

Conclusions

The widespread application of metallic NP-based products raises concerns about the safety of NPs. The brain is the most important organ that can be impaired by metallic NPs. Based on the vital role of PCD in neurological functions, we summarized articles related to the role of PCD in the neurotoxicity of metallic NPs, and we found that apoptosis was involved in the neurotoxicity of metallic NPs. Although autophagy is involved in nanotoxicity, few studies on the relationship between autophagy and neurotoxicity of metallic NPs have been reported. In addition, studies about the role of necroptosis or pyroptosis in the neurotoxicity of metallic NPs are scarce. Therefore, for unraveling the neurotoxic mechanisms underlying metallic NPs, the role of PCD in nanoneurotoxicity should be investigated comprehensively in the future.

Abbreviations

APAF1: Apoptotic protease-activating factor-1; BBB: Blood–brain barrier; cIAPs: Cellular inhibitor of apoptosis proteins; CMA: Chaperone-mediated autophagy; CTL: Cytotoxic T lymphocytes; Cu: Copper; CuO: Copper oxide; ER: Endoplasmic reticulum; FADD: Fas-associated death domain protein; HCECs: Human cerebral endothelial cells; HNE: Hydroxynoneal; HNPCs: Human embryonic neural precursor cells; IL-1β: Interleukin-1β; JNK: Jun N-terminal kinase; LAMP-2A: Lysosome-associated membrane protein type 2A; LTP: Long-term potentiation; MMP: Mitochondrial membrane potential; MWM: Morris water maze; NAC: N-acetylcysteine; NF-κB: Nuclear factor-κB; NK: Natural killer; N-MPG: N-(mercaptopropionyl)-glycine; NOX1: NADPH oxidase 1; NPs: Nanoparticles; NSCs: Neural stem cells; OS: Oxidative stress; PARP: Poly-ADP-ribose polymerase; PCD: Programmed cell death; RFK: Riboflavin kinase; RIP1: Receptor-interacting protein 1; ROS: Reactive oxygen species; TAB2: TAK1-binding protein 2; TAK1: Transforming growth factor-β activated kinase 1; TNFα: Tumor necrosis factor-α; TRADD: TNF receptor-associated death domain; TRAF2: TNF receptor-associated factor 2; USP21: Ubiquitin-specific peptidase 21; USPIO: Ultrasmall superparamagnetic iron oxide; ZnO: Zinc oxide

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Availability of Data and Materials

All the articles included in this review are obtained from Web of Science (2008-2016). The search terms include (1) apoptosis or autophagy or pyroptosis or necroptosis; (2) brain or "CNS" or neuro* or astrocyte or "neural stem" or microgli* or glia or neuroglia* or "central nerv*" or HT-22 or BV-2 or SH-SY5Y or PC12 or U87 or U251 or U373 or C6 or NEURO-2A or N9 or D384 or C17.2 or SK-N-SH or SHSY5Y or SKNSH or N27 or hippocamp* or hypothalam* or amygda* or cortex; (3) silver or "Ag" or iron or gold or "Au" or zinc or "Zn" or copper or "Cu" or alumina or "Al" or "ZnO" or "NiO" or "CuO" or "TiO_2" or "Al_2O_3" or "CeO" or Nanoceria or "Cerium oxide" or "Aluminum oxide" or "Zinc oxide" or "titanium dioxide" or "iron oxide" or "Fe_3O_4" or "Cu_2O" or "ZrO_2" or zirconia or "copper oxide" or "cobalt oxide" or metal or metallic; and (4) nano*.

Authors' Contributions
BS collected and reviewed the data and drafted the manuscript. BS, TZ, JL, and LQS helped in drafting the first version of the manuscript and in revisions. All authors read and approved the final manuscript.

Competing Interests
The authors declare that they have no competing interests.

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