

MINI-REVIEW

Nanoparticle Induced Oxidative Stress in Cancer Cells: Adding New Pieces to an Incomplete Jigsaw Puzzle

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Abstract

Nanotechnology is an emerging field with many promising applications in drug delivery systems. Because of outstanding developments in this field, rapidly increasing research is directed to the development of nanocarriers that may enhance the availability of drugs to the target sites. Substantial fraction of information has been added into the existing scientific literature focusing on the fact that nanoparticles usually generate reactive oxygen species to a greater extent than micro-sized particles. It is worth mentioning that oxidative stress regulates an array of cell signaling cascades that resulted in cancer cell damage. Accumulating experimental evidence over the years has shown that wide-ranging biological mechanisms are triggered by these NPs in cultured cells due to the unique properties of engineered nanoparticles. In this review, we have attempted to provide an overview of the signaling cascades that are activated by oxidative stress in cancer cells in response to different kinds of nanomaterials, including quantum dots, metallic and polymeric nanoparticles.

Keywords: Nanotechnology - oxidative stress - cancer - apoptosis

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Introduction

There is rapidly accumulating evidence that underscores the fact that nanoparticles induce oxidative stress that consequently results in apoptotic cell death of cancer cells. Increasingly it is being recognized that oxidative stress regulates an array of cell signaling cascades.

Reactive oxygen species (ROS) play a central role in a variety of cellular processes, such as cell cycle progression and apoptosis (Kim et al., 2013).

Apoptotic cell death has emerged as one of the most extensively studied biological phenomenon. Binding of ligands including TNF α , FasL or TRAIL to TNFR, Fas or DR respectively transduces the signals intracellularly. There is a well orchestrated mechanism that occurs at Death Domain (DD) containing receptor. FADD and pro-caspase-8 are assembled at death receptor, thus forming a Death Inducing Signaling Complex (DISC). However, cFLIP is an anti-apoptotic protein and has two death-effector domains (DEDs). cFLIP negatively regulates TNF, FasL or TRAIL induced signaling by interfering with the activation of caspase-8. Caspase-8 activates effector caspase-3. Caspase-8 also cleaves Bid to initiate intrinsic pathway. Truncated bid moves into mitochondrion to mediate release of cytochrome c, SMAC/DIABLO, OMI/HTRA. Apoptotic protease activating factor, procaspase-9 and cytochrome c assemble to form apoptosome that consequently regulates activation of caspase-3. In the

upcoming section we attempt to provide an overview of the signaling cascades, which are activated by oxidative stress in cancer cells. Nanoscience and nanotechnology have seen an exponential growth over the past decade largely due to the unique properties of engineered nanoparticles and wide-ranging biological mechanisms triggered by these NPs in cultured cells.

Cadmium Quantum Dots

Cadmium telluride quantum dots (CdTe-QDs) have recently been shown to effectively induce apoptosis in hepatocellular carcinoma HepG2 cells. In vitro assays revealed that CdTe-QDs triggered cell death in HepG2 cells was mediated through both extrinsic and intrinsic pathway. Mitogen-activated protein kinases (MAPKs) including JNK, Erk1/2 and p38 were found to be activated as a result of oxidative stress induced in HepG2 cells (Nguyen et al., 2013).

Similarly, cadmium sulfide quantum dots (bsCdS-QDs) stabilized with a biosurfactant was seem to prompt reactive oxygen species (ROS)-mediated apoptotic cell death in human prostate cancer LNCaP cells. The oxidative stress induced by these QDs is especially modulated by intracellular ROS generation, but it was further evidenced through increased levels of lipid peroxidation and enhanced activity of antioxidant enzymes such as superoxide dismutase and catalase. Higher activity

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of caspase-3 and caspase-9 was noted in QDs treated cancer cells. Finally, due to the induction of caspase-dependent apoptotic cell death of cancer cells, it is suggested that biologically stabilized CdS-QDs bear the potential to be applied in biomedicine, specifically in tumor therapy (Singh et al., 2012).

Cerium Oxide Nanoparticles

There is an exciting piece of evidence that suggests that Cerium oxide nanoparticles triggered apoptosis in hepatoma SMMC-7721 cells via ROS mediated activation of ERK1/2, JNK and p38 MAPK. Astonishingly, ROS scavengers dramatically reduced activated kinases and simultaneously there was a decrease in apoptotic rate (Chen et al., 2013).

Tungsten Carbide-Cobalt (WC-Co) Nanoparticles

Rapidly emerging information is adding new information into ever-expanding list of agents which have regulatory roles. In line with this approach, Tungsten carbide-cobalt (WC-Co) nanoparticles activity is studied in mouse epidermal cell line (JB6). The results suggested that there was transcriptional upregulation of miR-21 in WC-Co treated cells. To identify the pathway involved in WC-Co triggered activation of miR-21, ERK inhibitors and ROS scavengers were used. It was observed that ERK inhibitors considerably impaired WC-Co mediated expression of miR-21. Similarly, ROS scavengers also abrogated WC-Co triggered expression of miR-21 (Hou et al., 2013). Tungsten carbide-cobalt nanoparticles also induced oxidative stress in cancer cells. Oxidative stress induced cancer cells displayed notable release of cytochrome c from mitochondria thus highlighting involvement of intrinsic pathway in the induction of apoptosis (Zhao et al., 2013).

Aluminum Nanoparticles

Al₂O₃ nanoparticles are strong inducers of apoptosis in human mesenchymal stem cells. A recent study indicated that Al₂O₃ nanoparticles induced apoptosis via mitochondrial pathway. Moreover, there was a decrease in expression of anti-apoptotic protein Bcl-2 (Alshatwi et al., 2012).

Cobalt Nanoparticles

ROS generation was also evidenced in human hepatocarcinoma (HepG2) cells after treatment with cobalt oxide nanoparticles. Additionally, these nanocarriers elicited a significant reduction in glutathione with a concomitant increase in oxidative stress markers, such as, lipid hydroperoxide, superoxide dismutase, and catalase activity. Altogether, the induction of these toxicological processes appeared to be the mechanisms underlying DNA damage and apoptosis. The apoptotic activity of these nanoparticles was especially proven by the increased level of caspase-3, which plays a vital role in both initiation and

execution of apoptosis. (Alarifi et al., 2013a). There is a recent exciting piece of evidence focusing on Chitosan-coated cobalt oxide nanoparticles mediated apoptosis in Jurkat cells via generation of ROS. Additionally it was noted that nanoparticles stimulated secretion of TNF α (Chattopadhyay et al., 2014).

Selenium Nanoparticles

Selenium nanoparticles modified with folic acid (FA-Se-NPs) were reported to enter into mitochondrial compartments of human breast cancer cells (MCF-7), which in turn induced ROS generation and finally led to the damage of mitochondria. Those changes then induced the activation of caspase-9 and of downstream apoptosis executor caspase-3, which further induced mitochondria-dependent apoptosis. Based on the mechanisms of FA-Se-NPs toxicity, the authors stated that these nanocarriers could be targeted to tumor cells thus providing a unique opportunity to explore potential of these nanoparticles in targeted therapy (Pi et al., 2013).

Titanium Dioxide (TiO₂) Nanoparticles

There is a direct piece of evidence that suggests that TiO₂ nanoparticles induced cytotoxicity was Bax/Bak independent. Proof of the concept was obtained via cell death signaling analysis in titanium dioxide nanoparticles treated Bax/Bak deficient cells. These nanoparticles induced cell death in Bax/Bak deficient cells thus highlighting the fact that there is an alternate mechanism opted. Surprisingly, these nanoparticles colocalized with lysosomes in transformed cells. It was concluded that TiO₂ nanoparticles in transformed cells induced lysosome-mediated cell death (Zhu et al., 2012). However another contemporary study revealed that titanium oxide induced apoptotic cell death. Bax and Fas were found to be upregulated in nanoparticle treated cells (Yoo et al., 2012).

Recently, it was reported that TiO₂ nanoparticles induced oxidative stress in A549 cells. Oxidative stress was associated with ROS and malondialdehyde (MDA) generation, together with a decrease in the activity of catalase and glutathione. Notably enhanced expression of p53, p21 and cleaved caspase-3, while Bcl-2 was suppressed at both mRNA and protein levels (Srivastava et al., 2013).

Nickel Oxide Nanoparticles

Nickel oxide nanoparticles were demonstrated to induce cytotoxic responses in HepG2 cells via apoptotic pathway, being the ROS generation and, thus, the oxidative stress, the mechanism underlying this process. Studies of the expression level of mRNA of apoptotic genes suggested that these nanoparticles exerted their cytotoxic effects via ROS. Apoptosis was mediated through Bax/Bcl-2 pathway (Ahamed et al., 2013).

On the other hand, nickel ferrite nanoparticles were also found to induce oxidative stress in human lung epithelial cancer (A549) cells, which was evidenced by ROS generation and glutathione (GSH) depletion. These

oxidative processes, especially the ROS generation, appeared to be the mechanism underlying apoptosis. Nanoparticles treated cancer cells displayed an increase in Bax, caspase-3 and caspase-9 expression, whereas survivin and Bcl-2 were down-regulated (Ahamed et al., 2011).

Cuprous Oxide Nanoparticles

Cuprous oxide nanoparticles are also notable inducers of apoptosis in cancer cells. It was shown that these nanoparticles were taken up by mitochondria that consequently resulted in damage of membrane and release of pro-apoptotic proteins into cytosol (Wang et al, 2012).

Silver Nanoparticles

Silver nanoparticles have been shown to effectively induce apoptosis in squamous cell carcinoma via TLR2. It is worth mentioning that targeted inhibition of TLR-2 substantially reduced nanoparticle mediated cell death (Kim et al, 2012). Silver nanoparticles have also been shown to strongly induce apoptosis in murine dendritic cell line in a ROS dependent manner (Kang et al, 2012).

The cytotoxicity of silver nanoparticles in lung cancer (A549) cells revealed a strong correlation between the levels of ROS and mitochondrial damage or early apoptosis. Moreover, ROS generation also appeared to be a mediator of genotoxicity. Finally, it was demonstrated that a pretreatment with an antioxidant greatly decreased the cytotoxic potential of such nanocarriers (Foldbjerg et al., 2011).

Silver nanoparticles coated with poly vinyl pyrrolidone (PVP) also enhanced ROS levels in a human monocytic leukemia (THP-1) cell line. Moreover, the initiation of apoptosis cell death as a function of dose and exposure time implied the presence of a ROS-mediated apoptotic process (Foldbjerg et al., 2009). However, surprisingly, Nanosilver-exposed HepG2 and Caco2 cells did not represent any notable oxidative stress, as evidenced by the data obtained through dichlorofluorescein assay (Sahu et al., 2014).

Finally, silver nanoparticles displayed cytotoxicity against MDA-MB-231 breast cancer cells in dose-dependent manner. Caspase-3 activation and ROS generation resulted in induction of apoptosis. Altogether, these mechanisms led to induction of apoptosis, which was further confirmed through resulting nuclear fragmentation. The apoptotic mode of cell death gives reason to consider the silver nanoparticles as a potential alternative agent for human breast cancer therapy (Gurunathan et al., 2013).

Zinc nanoparticles

Zinc oxide (ZnO) nanoparticles effectively induced oxidative stress in A375 cells, as evidenced by ROS generation. Higher lipid peroxidation rate and depleted glutathione levels were noted (Alarifi et al., 2013b).

Additionally, Sharma et al. (2012) also evidenced that the mode of cell death induced by ZnO nanoparticles

in HepG2 cells was apoptosis. The oxidative stress appeared to be a key mechanism of toxicity induced by these nanocarriers and it was also observed that the ROS triggered a decrease in mitochondria membrane potential and an increase in the ratio of Bax/Bcl-2, which led to mitochondria mediated pathway involved in apoptosis. In addition, it was revealed that these ZnO nanoparticles activated JNK, p38 MAPK. However, apoptosis was found to be independent of JNK and p38 pathways. Similarly, Akhtar et al. (2012) demonstrated that ZnO nanoparticles selectively induced apoptosis in cancer cells, which was likely to be mediated by ROS via p53 pathway.

Induction of oxidative stress in human amnion epithelial (WISH) cells, evidenced by significant intracellular ROS production, was also attributed to cell treatment with zinc ferrite nanoparticles. Through the confirmation of some damage in the inner mitochondrial membrane integrity, it was revealed that the ROS generation led to apoptosis. The proof of this concept was obtained through the up-regulated expression levels of tumor suppressor p53, caspase-3 and pro-apoptotic Bax genes, and down-regulated expression levels of the anti-apoptotic gene Bcl-2. Altogether, the results obtained demonstrated that the nanoparticles triggered apoptosis via ROS and through mitochondria dependent intrinsic apoptotic pathway (Saqib et al., 2013).

Silica Nanoparticles

Silica nanoparticles offer new perspectives in medicine and can be applied in biosensor, drug delivery and cancer therapy. There is a recent report suggesting toxicological effects of silica (E551) particles frequently used as anti-caking agent in food products. It was shown that high concentration of E551 exerted cytotoxic effects by enhancing ROS in WI-38 cells (Athinarayanan et al., 2014). Silica oxide (SiO₂) nanoparticles mediated a cytokine inflammatory response and induced oxidative stress in peripheral blood mononuclear cells (PBMC) (Mendoza et al., 2014). Mesoporous silica nanoparticles have also shown considerably improved delivery of drugs intracellularly, which consequently kill cancer cells with enhanced efficacy. It was stated that the mechanisms underlying cancer cell death included increased necrosis, due to augmented oxidative stress, and apoptosis (Li et al., 2014).

However, it was demonstrated that they induced cytotoxicity and apoptosis in many cell types, such as human epithelial carcinoma (A431) cell line and in lung cancer (A549) cells. These cytotoxic effects were likely to be mediated through ROS generation and oxidative stress, which in turn led to apoptosis via intrinsic pathway. The mRNA levels of caspase-3 and caspase-9 genes was up-regulated in a dose-dependent manner in both cell types, which proved the occurrence of apoptotic cell death due to silica nanoparticles exposure (Ahmad et al., 2012). On the other hand, it was shown that the silica nanoparticles exerted their inhibitory effects by interfering with the MAPK/ERK1/2 as well as the Nrf2/ARE signaling pathways (Gehrke et al., 2013).

Gold Nanoparticles

It was reported that gold nanoparticles can enhance apoptosis of human breast cancer MCF-7 cells in dose dependent manner, suggesting the potential application of these nanocarriers in the cancer therapy (Selim and Hendi, 2012). The apoptotic effects were examined by nuclear DNA staining assay, being the changes in nuclei observed under ultraviolet fluorescent microscope. Moreover, quantitative real-time PCR analysis displayed that mRNA levels involved in the apoptosis was altered by these nanoparticles, suggesting that they may induce apoptosis in cancer cells via p53, bax/bcl-2 and caspase pathways.

Polymeric Nanoparticles

A chitosan-based nanodevice designed to carry low concentration of silver nanoparticles was reported as an efficient approach to be use in cancer therapy. These nanoparticles efficiently induced apoptosis in human colon cancer (HT 29) cells, via intrinsic apoptotic pathway and increased production of intracellular ROS (Sanpui et al., 2011).

More recently, saturated shell core-shell tecto-dendrimers, using amine-terminated polyamidoamine (PAMAM) generation 5 (G5) as core and carboxyl-terminated PAMAM G2.5 as shell (G5G2.5 tectodendrimers), were synthesized and evaluated for their mechanisms of toxicity in human melanoma cells. G5G2.5 cytotoxicity was related to the initiation of oxidative stress, evidenced by a rapid depletion of intracellular levels of glutathione, which in turn was linked to the induction of apoptosis. In conclusion, the increase in the percentage of non-viable cells suggests that G5G2.5 toxicity might display an important antimelanoma activity (Schilrreff et al., 2012). Finally, a recent review of Mirakabad et al. (2014) report on poly (lactic-co-glycolic acid) (PLGA) nanoparticles as one of the most effective biodegradable polymeric delivery systems for anticancer therapy. Many antitumor drugs have been encapsulated in PLGA nanocarriers, which provide promising strategies for cancer treatment.

Conclusions

There is considerable progress regarding comprehension of the mechanisms that regulate cell death pathways, including especially the understanding about oxidative stress and its resulting negative effects to the cells. This knowledge has offered new perspectives researchers to cancer scientists to target these pathways as an anticancer approach. In this context, the present paper reviews the master regulators of nanoparticle-induced oxidative stress in cancer cells. Noteworthy is that the oxidative stress initialization can be attributed to the oxidative properties of the nanoparticles themselves and/or to oxidant generation upon their interaction with cellular material. Reactive oxygen species generation and oxidative stress occur as early events leading to nanomaterial-induced injury. These mechanisms are important factors in the apoptosis process, in DNA

damage, inflammation and many other cellular processes, which together can be related to the nanoparticle antitumor activity.

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