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Potential of Zinc Oxide Nanoparticles as an Anticancer Agent: A Review

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ABSTRACT

According to reports, one of the leading causes of mortality is cancer. Over the years, numerous approaches have been devised to lessen chronic pain and death as well as to elevate the quality of life. However, a scarcity persists in the effectiveness of cancer treatments. Early cancer identification and medication delivery with excellent specificity to reduce toxicities are two critical elements in ensuring effective cancer treatment. As a result of severe systemic toxicities and issues with current cancer diagnostic and treatment procedures, alternative nanotechnology-based techniques are being employed to improve detection and minimize disease severity. Nanotechnology has shown promising breakthroughs in cancer therapy by eliminating tumours with minimal damage to surrounding healthy cells. Since zinc is one of the necessary trace elements found in large amounts in human body tissues, zinc oxide nanoparticles (ZnO NPs) are said to be the most cost-effective and have the least hazardous characteristics of all metal oxide nanoparticles. In addition, ZnO NPs have several biological uses, notably in the field of drug administration. In this review, we tried to explore the advantage of ZnO NPs in the biomedical field, particularly in the treatment of cancer which can help to facilitate future research progress.

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1 Introduction

Nanoscience and nanotechnology refer to a rapidly growing field of study that includes structures, technologies, and systems having unique features and functionalities attributing to the positioning of their size on the 1–100 nm scale. This area of research attained promising awareness among the public and controversies in the early 2000s and commenced commercial applications. Nanotechnologies have a huge impact on biology, chemistry, materials science, and physics (Bayda et al. 2019). Nanomaterials' unique size-dependent property makes them essential in a variety of fields, including material sciences, medical and diagnostics, agriculture, and cosmetics (Salata 2004). Scientists have been drawn to various nanomaterials such as zinc oxide nanoparticles (ZnO NPs), gold nanoparticles (Au NPs), copper oxide nanoparticles (CuO NPs), titanium dioxide nanoparticles (TiO_2 NPs), and silver nanoparticles (Ag NPs) as a result of their distinct optical and chemical reactions that may be changed by changing the shape in various ways (Yaqoob et al. 2020). Further, the role of ZnO NPs in the pharmaceutical industry is also well known and these have antimicrobial, acaricidal, larvicidal, and anti-diabetic activities (Bala et al. 2016). In this review, we tried to explore the advantage of ZnO NPs in the treatment of cancer which can facilitate the future research program.

2 Nanotechnologies in Therapeutic Application

Nanotechnology has been used in diagnostics and molecular imaging with promising results. Cancer biomarkers such as cancer-linked proteins, circulating tumour DNA and cells, and exosomes can be detected using nanoparticles (Jia et al. 2017). In addition, visualizing tumour tissue with nanoparticles has allowed for cancer diagnosis in its preliminary phases. The metastases in lung cancer may be identified by producing superparamagnetic iron oxide nanoparticles (SPIONs) that can be employed in MRI imaging of the cancer cells as the SPIONs' target (Wan et al. 2016).

Nanotechnologies can assist physicians to diagnose at the cellular and molecular levels. Moreover, nanotechnologies further assist in point of care diagnostics, therapeutics, and the development of medicine (Riehemann et al. 2009). Many nanomaterials-based drug delivery systems including liposomes, nanomicelles, branched dendrimers, nanostructured lipid formulations, and nanoconjugates have been developed. The nanomaterials-based drug delivery systems offer present various advantages such as nontoxicity, biocompatibility, good biodegradability, and anti-inflammatory (Barani et al. 2021). For instance, liposomes can be PEGylated to extend the circulation shelf duration and prevent their elimination by the mononuclear phagocytic system (Suk et al. 2015). Qi et al. (2018) examined the efficiency of paclitaxel containing PEGylated liposomal nanoformulation (PL-PTX) in suppressing the multiplication of ovarian carcinoma cells *in vivo* and *in vitro* models. The PL-PTX-mediated treatment markedly inhibited the aggressiveness and growth of ovarian tumor cells.

The nanoparticles (NPs) used in medical treatment typically have specific sizes, shapes, and surface characteristics because these three factors have a significant impact on the effectiveness of nano-drug delivery and therefore regulate therapeutic efficacy (Bahrami et al. 2017). NPs with diameters ranging from 10 to 100 nm are commonly used in cancer therapy since they can efficiently implement drug delivery while also achieving enhanced permeability and retention (EPR) (Greish 2012). Nano-carriers in the treatment of cancer target tumour cells after absorption via the carrier effect, targeting substance, and release of the drugs into the target tumour cells to initiate cytotoxicity (Senapati et al. 2018). Meanwhile, the targeting system shields normal cells from drug cytotoxicity, which helps to mitigate the side effects of cancer (Bahrami et al. 2017).

The potential benefits of nanobiotechnologies raise great hopes in therapies for cancer and chronic lung diseases, antimicrobial agents, diabetes, and gene therapy. Metal nanoparticles, such as gold, silver, copper, and zinc nanoparticles, have been extensively studied due to their unique chemical and optical characteristics (Maslanka Figueroa et al. 2021). For instance, due to their propensity to scatter visible light, gold nanoparticles have been utilized as contrast agents in cancer detection and therapy (Kolhe and Parikh 2012). On the other hand, silver nanoparticles have attracted the interest of biomedical researchers due to their high inherent antibacterial efficacy and non-toxicity. Among the numerous possible uses of silver nanoparticles, much attention and effort have been focused on their prospective implications in wound dressing, tissue scaffolding, and protective clothing applications (Gudikandula et al. 2017). Copper has inherent antibacterial and anti-inflammatory properties, making copper-based nanomaterials a good candidate for designing microbe-resistant medical devices (Verma and Kumar 2019). Whereas ZnO nanostructures have high catalytic efficiency as well as a great adsorption ability and are more widely utilized in the production of sunscreens (Sabir et al. 2014). The characteristics of nanomaterials, such as shape, size, surface structure and charge, chemical composition, agglomeration, aggregation, and solubility, can impact how nanoparticles interact with biomolecules and cells. Biomolecules such as protein, cell surface receptors, DNA, cell membrane, and haemoglobin have similar diameters to metal nanoparticles. As a result, nanoparticles with a diameter within 50 nm have been demonstrated to interact with human cells both extracellularly and intracellularly (Barua and Mitragotri 2014).

3 Application of Nanotechnology in Cancer

Chemotherapy, radiation therapy, and surgery have long been recognized as the primary cancer treatment options. On the other

hand, these therapy approaches have a slew of negative side effects owing to their non-selective effects on cells, which means they may harm healthy cells as well. As a result, it's critical to create therapies that can kill cancer cells with minimal or no side effects to normal cells. Although metals and metal ions can control a variety of biological processes in live creatures, larger dosages of metal or metal ions supplied externally for therapeutic or diagnostic purposes might be harmful. Furthermore, it has been observed that the usage of bulk materials in medicine has disadvantages such as non-specificity and low bioavailability (Rizvi and Saleh 2018). In this light, scientists predicted that converting bulk chemicals to nanoparticulate forms could change the materials' physicochemical and biological characteristics, therefore minimizing the disadvantages (Jeevanandam et al. 2018).

Cancer nanotechnology is a branch of nanotechnology that combines many fields, including material science and physics, as well as cancer biology. This multidisciplinary collaboration has resulted in the development of devices or materials with critical components ranging from 1 to 100 nanometers, allowing for advancements in cancer treatments and tumour imaging. This developing field of nanohealth might enable the early identification of human tumours, regardless of where the original tumour or metastases are located, as well as ways to efficiently eliminate tumours and their accompanying vascular supply with minimum consequences (Zhang et al. 2019). According to Wu et al. (2013), the pH response to fluorescent nanoprobes can assist in the detection of fibroblast activated protein-a on the cell membrane of fibroblasts associated with tumours. Furthermore, after being absorbed, nanocarriers in cancer therapy target tumour cells via the carrier impact of nanoparticles and the positioning effect of the targeted material. The medications are then delivered to tumour cells to destroy them (Chen et al. 2010). Due to their size and surface features, nanocarriers can elevate permeability and retention which lead to the enhanced half-life of medications and cause their accumulation in tumour tissues (Bertrand et al. 2014; Kalyane et al. 2019).

3.1 Zinc Oxide Nanoparticles in Cancer Therapy

ZnO NPs have a molecular weight of 81.38 g/mol and appear as white odourless powder. It has a wurtzite crystal structure and is a semiconductor with a large band gap (3.37 eV at ambient temperature). The tiny size of ZnO NPs allows zinc to be absorbed more easily by the body. Zinc stands out among metals due to its strong reducing potential, mild reactivity, and five stable isotopes, as well as its wide range of applications and environmental suitability (Keerthana and Kumar 2020). ZnO NPs' easier synthesis and functionalization due to the presence of -OH group, biodegradability, biocompatibility, multiple loading of drugs or therapeutic molecules, and economical biodegradability and low toxicity have enhanced their use in cancer drug delivery more than other nanoparticles (Mocchegiani et al. 2013).

Since zinc oxide does not interact with key pharmacological active ingredients, it is an excellent option for drug delivery (Beyene et al. 2021). ZnO NPs were found to be capable of targeting multiple cancer cell types as well as inhibiting cancer cell proliferation, sensitizing drug-resistant cancer, preventing cancer recurrence and metastasis, and reviving cancer immune surveillance (Wang et al. 2017; Moghaddam et al. 2017; Aalami et al. 2020). The use of NPs in targeted medication delivery has been a promising area of study for cancer therapy. The number of medicines used to treat malignant cells will be reduced. Due to the intricacy of cancer, an ideal anticancer treatment would target cancer cells and the tumour microenvironment.

Combining conventional cancer treatments with NPs is one way to minimize their toxicity (Vinardell and Mitjans 2015). *In vitro* combination of ZnO NPs with paclitaxel, cisplatin or daunorubicin improves the efficacy of these chemotherapeutics (Hackenberg et al. 2012). Different-sized ZnO NPs may substantially increase drug targeting and accretion of daunorubicin in leukemia cancer cells and therefore function as an effective agent to improve drug delivery (Guo et al. 2008). In a similar study, it was reported that loading doxorubicin, an anticancer medication, inside ZnO NPs resulted in greater anticancer activity than either ZnO NPs or doxorubicin alone. It was believed that the drug's cytotoxic potential was enhanced as a result of the synergy created by the drug's anticancer effect combined with that of ZnO NPs (Sharma et al. 2016).

The Food and Drug Administration (FDA) has authorized zinc oxide nanoparticles as a new and effective anticancer treatment (Anvarinezhad et al. 2020). In addition to producing reactive oxygen species (ROS), ZnO NPs can cause selective cytotoxicity against cancer cells by inducing disequilibrium of zinc-dependent protein activity (Table 1) (Wahab et al. 2014). ZnO NPs harm cancer cell lipids, protein, and nucleic acid when there are high amounts of ROS and oxidative stress (Valko et al. 2006) (Figure 1). Elevated ROS can damage cell membranes by protein denaturation and lipid peroxidation, resulting in necrosis, and DNA damage, culminating in apoptosis (Wang et al. 2017). Highly reactive ROS species can react with DNA components, changing DNA composition and causing mutations. The OH radical, a highly reactive oxygen species, induces single-stranded DNA breaking (Phaniendra et al. 2015). These DNA breaks and crosslinks cause DNA damage, which activates a mitochondrial apoptotic pathway, resulting in cell death by apoptosis (Mishra et al. 2017). The primary mechanism of cell death in this cytotoxic reaction of ZnO NPs is thought to be apoptosis, or programmed cell death (Bisht and Rayamajhi 2016).

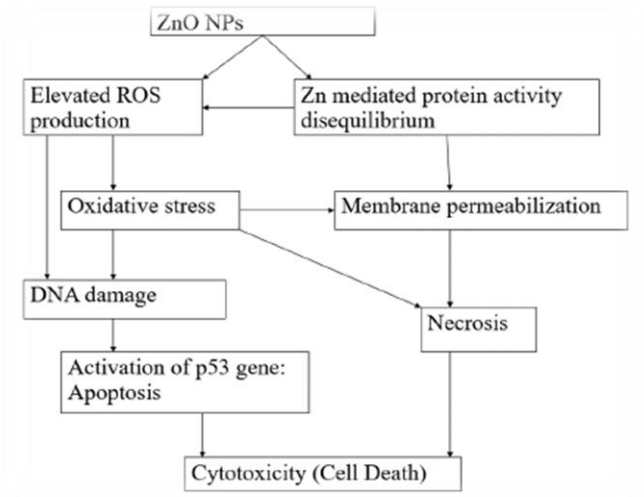

Figure 1 A schematic outline of the mechanism of cytotoxicity of ZnO NPs which leads to cell death (Bisht and Rayamajhi 2016).

Table 1 Anticancer activity of ZnO NPs on different types of cancer cell lines

Cancer cell line	Key observations	Reference(s)
Human ovarian cancer cells (SKOV3)	Dose-dependent loss of cell viability and presence of characteristic apoptotic features such as loss of adherence to enhanced ROS generation and loss of mitochondrial membrane potential	Bai et al. 2017
Human epidermal carcinoma cell line (A431)	Generation of ROS and cell cycle arrest in S and G2/M phase with the higher uptake in G2 M phase compared with other phases	Patel et al. 2016
Human liver hepatocellular carcinoma (Hep-G2)	Dose-dependent cytopathic effects, caspase-3 activation, and apoptosis	Chung et al. 2015
Human breast cancer cell line (MCF-7)	Disequilibrium of zinc-dependent protein activity	Wahab et al., 2014
Human pulmonary adenocarcinoma cell line (LTEP-a-2)	Increase in ROS coincided with depletion of GSH in apoptotic cells, suggesting that oxidative stress may be the primary toxicological mechanism	Wang et al., 2015
Human neuroblastoma cells (SHSY5Y)	ZnO NPs induced significant cytotoxicity in a size-dependent manner and reactive oxygen species generation was the main factor that lead to a decrease in cell viability and apoptosis	Liu et al., 2017
Human cervical adenocarcinoma cells (HeLa)	mRNA expression of apoptotic gene p53 and level of ROS increased in a dose-dependent manner	Pandurangan et al., 2016
Human skin melanoma (A375)	Significant decrease in cell viability and generation of ROS. Apoptosis confirmed by chromosomal condensation assay and caspase-3 activation	Alarifi et al., 2013; Mishra et al., 2017
Human epithelial colorectal adenocarcinoma (Caco-2)	Significant reduction in glutathione and increase in ROS and lactate dehydrogenase	Kang et al., 2013; Song et al., 2014

According to previous studies, ZnO NPs might be used as a pH-dependent medication carrier with improved cellular absorption and tumour spheroid penetration (Muhammad et al. 2011; Zare et al. 2019). ZnO NPs have been shown to efficiently inhibit cell adhesion, migration, and carcinogenesis in cancer (stem-like) cells, as well as sensitize medication therapy. According to these findings, ZnO NPs offer a lot of promise as a multifunctional nanomedicine for anticancer research (Hamrayev et al. 2020).

Nanomedicine is still a technology-driven field with many scientific difficulties ahead of it. It does, however, represent a developing sector with the potential to meet the long-standing demand for new and improved anti-cancer medicines. According to Rasmussen et al. (2010), NPs are not always similar, different from batch to batch, and they may change in surface chemistry or size distribution and thus eliciting diverse biological responses. There is currently inadequate *in vivo* evidence to determine the biological impact of these materials on inflammation and functional modifications at the cellular or whole-body level. ZnO NPs can accumulate in the body and induce organ toxicity or disintegration in unanticipated ways. The high solubility of the particles was attributed to the harmful consequences, which

included cytotoxicity and mitochondrial dysfunction. There is a need to assess if the potential benefits of these nanomedicines exceed the possible risks associated with nanotoxicity (Condello et al. 2016). ZnO NPs have shown remarkable potential in cancer detection and therapy; nevertheless, further in-depth and sophisticated study of ZnO NPs, extensive knowledge of the cellular and molecular processes, and clinical trials are necessary in the future for improved cancer theranostic outcomes and to assess their long-term health concerns (Anjum et al. 2021).

3.2 The Advantages of Utilization of Zinc Oxide Nanoparticles

ZnO NPs can be synthesized via physical, chemical, and biological methods or top-down or bottom-up processes. For the production of NPs, the green synthesis technique is more advantageous than other traditional methods (Gour and Jain 2019). This is owing to its environmentally responsible strategy, which avoids the use of harmful chemicals. Furthermore, this technique does not need high pressure or temperature. The production of ZnO NPs using environmentally friendly ingredients such as plant extract, bacteria, fungus, and enzymes offer advantages in terms of compatibility with pharmaceutical and other biomedical applications (Kalpana and Devi Rajeswari 2018). The bottom-up method of nanoparticle biosynthesis is based on the reduction/oxidation process. Metal compounds are reduced into nanoparticles by microbial enzymes or plant phytochemicals with antioxidant or reducing capabilities (Ovais et al. 2018). For the method to work, the solvent medium utilized for synthesis, the choice of an eco-friendly reducing agent, and a non-toxic substance for nanoparticle stabilization are also critical (Gour and Jain 2019).

Another characteristic of ZnO NPs is their ability to produce ROS, which can cause cell death when the level of ROS exceeds the cell's antioxidant capacity (Ancona et al. 2018). Zinc is an essential cofactor in a variety of cellular mechanisms. This element is crucial in the regulation of enzymes in the body that are involved in the synthesis and degradation of lipids and proteins, thereby maintaining homeostasis (Skrajnowska and Bobrowska-Korczak 2019). Furthermore, the neutral hydroxyl groups of ZnO NPs can be easily functionalized by a variety of biocompatible molecules and thus increasing their biocompatibility (Abdelmigid et al. 2022).

Aside from specificity, targeted NPs provide other therapeutic benefits such as multidrug conjugation, simple release kinetics adjustment, selective localization, and bypassing multidrug resistance mechanisms (Jiang et al. 2018). Many functionalizations and surface modification strategies to reduce unwanted toxicity and improve the biocompatibility of NPs have been studied (Kim et al. 2017). For instance, surface functionalization of ZnO NPs can be done with various types of biological molecules, such as proteins, peptides, and nucleic acids (Namvar et al. 2016; Othman et al. 2016). The anticancer action of ZnO NPs is unaffected by the biocompatible coating, but the targeting effects on cancer cells are enhanced, and the safety against normal cells is improved (Jiang et al. 2018).

Namvar et al. (2016) used green synthesis to create a hyaluronan-ZnO nanocomposite (HA/ZnO) for cancer therapy. In a dose- and time-dependent manner, the HA/ZnO nanocomposites produced morphological alterations and reduced the growth of pancreatic adenocarcinoma PANC-1 cell, ovarian adenocarcinoma CaOV-3 cell, colonic adenocarcinoma COLO205 cell, and acute promyelocytic leukemia HL-60 cell. The normal human lung fibroblast (MRC-5) cell line did not show any toxicity after being exposed to the HA/ZnO nanocomposite for 72 hours. In another research, peptide-conjugated ZnO NPs were shown to improve the targeting effects of ZnO NPs on MDA-MB-231 cells at lower dosages (Othman et al. 2016). Also, when HT29 cells were treated with peptide ZnO NPs conjugates, there was an increase in cytotoxicity compared to ZnO NPs or peptide therapy alone (Bai Aswathanarayan et al. 2018). This indicates that functionalizing NPs improves their ability to kill cancer cells, making them a promising class of nanodrugs for cancer treatment (Bai Aswathanarayan et al. 2018).

Conclusion

Nanotechnology has had a transformative influence on biomedicine, with remarkable progress over the previous several decades. Nanomaterials can display characteristics unique from molecules and bulk solids, and enable novel interactions with biomolecules on the surface and inside cells. Because of their intrinsic capacity to promote ROS production and trigger apoptosis, ZnO NPs, like other metal oxides NPs, offer great medicinal potential. ZnO NPs have these properties, making them effective anticancer and antimicrobial agents. ZnO nanoparticles are ideal candidates as biodegradable, biocompatible, "deliver and dissolve" platforms for cancer therapy because of their numerous appealing physicochemical characteristics and huge promise for different biomedical applications. When loaded and given with other medicinal treatments, ZnO NPs have been shown to have synergistic effects. Given the future potential of ZnO NPs, a greater understanding of their toxicity is crucial. Over the next few decades, it is predicted that research into biomedical uses of ZnO NPs would thrive, with new advances in this burgeoning field.

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