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EXPLORING THE ANTITUMOR ACTIVITIES OF ZINC OXIDE NANOPARTICLES

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ABSTRACT

The field of cancer treatment is plagued by serious obstacles, which led to the study of new therapeutic strategies. Zinc oxide nanoparticles (ZnO NPs) are a potential cancer treatment agent, which has received considerable attention for its unique properties and potential cancer resistance properties. The following is a comprehensive analysis of current research on the anti-tumor properties of ZnO NPs. It starts with a discussion of the characterization techniques and synthesis methods used in the production and examination of ZnO NPs. After that, we investigate the mechanisms that are responsible for their anti-tumor properties, including their ability to produce reactive oxygen species (ROSs) and stimulate apoptosis in cancerous cells. In addition, in vitro and in vivo studies are reviewed to evaluate the effectiveness of ZnONP against a variety of cancers. Despite their promising nature, barriers such as bioavailability and toxicity must be overcome before clinical implementation. The review concludes with a discussion on the potential applications and future prospects of ZnO NP in combination therapy and targeted drug delivery systems. The aim of this study is to provide a detailed analysis of current research on ZnO NP in cancer therapy and to provide guidance for future research in this blessed field.

KEYWORDS: Zinc oxide nanoparticles, cancer therapy, antitumor efficacy, combination therapy, targeted delivery, precision medicine.

INTRODUCTION

Contemporary cancer treatments, including targeted therapy, radiation therapy, surgery, chemotherapy and chemotherapy, have shown better patient prognosis, but are often hampered by resistance development and adverse side effects.[1] In view of these obstacles, nanotechnology is emerging as a potential profitable field in cancer treatment. The unique advantages of nanoparticles in drug delivery, imaging and therapy stem from their small dimensions and malleable properties.[2] Zinc oxide nanoparticles (ZnO NPs) have received considerable attention for their potential as a cancer treatment. Zinc oxide nanoparticles (ZnO NPs) have several useful characteristics, including biocompatibility, easy synthesis, and the ability to produce reactive oxygen species (ROSs).[3] Research into the potential antitumor effects of ZnO NP is motivated by its unique physicochemical properties and its interaction with biological systems. ZnO NPs, unlike conventional therapies, have the ability to selectively target cancer cells while minimising damage to healthy tissues. In addition, their ability to stimulate apoptotic cell death and ROS-mediated cytotoxicity in cancer cells make them attractive competitors for cancer therapy.[4] This article is intended to conduct an extensive investigation into the antitumor properties of ZnO NPs. The synthesis methods and characterization techniques used in the production and analysis of ZnO NPs will be thoroughly studied. Furthermore, we will explain the mechanisms that regulate their antitumor properties, including the pathways of apoptosis and ROS production.[5] Through a review of both in vitro and in vivo studies, we will assess the efficacy of ZnO NPs against various cancer types. Additionally, we will address the challenges and limitations hindering the clinical translation of ZnO NPs and suggest future research directions. By consolidating current knowledge, this review seeks to contribute to the advancement of ZnO NPs as a promising avenue for

cancer therapy.[6]

Overview of traditional cancer treatment methods

Traditional cancer treatment methods encompass a range of approaches aimed at targeting and eliminating cancerous cells in the body.[7] A wide range of therapeutic approaches are utilised in the management of cancer, including but not limited to surgery, chemotherapy, radiation therapy, and hormone therapy. Surgical intervention involves the invasive removal of tumours and surrounding tissue, with the ultimate goal of completely eliminating the malignant development.[8] Chemotherapy utilises pharmaceutical agents to eliminate or hinder the growth of cancer cells by means of direct damage or interference with their reproductive capacity. By inducing DNA damage and impeding further growth, radiation therapy eliminates cancer cells or reduces the size of tumours through the use of high-energy radiation beams.[9] On the contrary, hormone therapy operates by impeding the production or operation of particular hormones that promote the development of hormone-sensitive malignancies, including prostate and breast cancer. These conventional treatment approaches may be implemented singly or in combination, depending on the type, stage, and specific patient factors of the malignancy.[10] While these techniques have been crucial in the treatment of various types of cancer and the improvement of patient outcomes, they are not without their drawbacks and limitations. Continuous research endeavours strive to develop innovative, enhanced therapies that exhibit higher levels of precision, efficacy, and tolerability. These therapies encompass targeted therapy and immunotherapy, and serve to advance the field of cancer treatment.[11]

Synthesis Methods of ZnO Nanoparticles

Zinc oxide nanoparticles (ZnO NPs) can be synthesized through various methods, each offering distinct advantages and challenges. These synthesis methods can be broadly categorized into physical, chemical, and biological approaches.[12]

A. Physical Methods

Physical techniques for producing ZnO NPs encompass various procedures that convert bulk ZnO into nanoparticles: laser ablation, mechanical milling, vapour condensation, and mechanical milling. These techniques provide meticulous regulation of particle dimensions, structure, and purity.[13]

1. Vapor Condensation

Vapour condensation is a widely used and versatile method for synthesis of nanoparticles, and size, shape, and morphology can be precisely controlled. This approach involves condensation of vapour phase precursors on a substrate or nucleation site, which is facilitated by controlled parameters, including temperature, pressure and gas composition. As a result, nanoparticle formation begins.[14] The method of vapour condensation consists of various submethods, such as chemical vapour deposits (CVD), physical vapour deposits (PVD) and evaporation condensation processes. Each of these sub-methods provides unique benefits and practical applications in nanoparticle synthesis.[15] Physical liquid deposition facilitates the production of nanoparticles by vaporizing solid precursor materials by thermal evaporation, sputtering or laser ablation, and then redirecting the liquid deposition that forms on a substrate to initiate and expand.[16] Due to its ability to regulate the size and composition of nanoparticles with extreme precision, this method is well suited for the synthesis of metal nanoparticles, semiconductors, and ceramics that are useful in the fields of electronics, catalysis, and sensing.[17] On the other hand, chemical vapour deposition uses nucleation processes and chemical reactions to produce nanoparticles from gas precursor molecules in the presence of a substrate. This method offers excellent control over nanoparticle composition, uniformity, and crystallinity and is commonly employed for synthesizing semiconductor nanomaterials, thin films, and coatings with tailored properties for photovoltaic, optoelectronic, and microelectronic applications.[18] Evaporation-condensation processes involve the evaporation of precursor materials followed by rapid cooling and condensation onto a substrate, leading to the formation of nanoparticles with controlled size and morphology.[19] This method is particularly suitable for synthesizing nanoparticles of refractory metals, oxides, and alloys with high melting points and thermal stability. Vapor condensation techniques offer several advantages such as scalability, reproducibility, and tunability, making them attractive for large-scale production of nanoparticles for industrial applications.[20] However, challenges such as substrate compatibility, precursor purity, and control over nanoparticle size distribution need to be addressed to optimize the synthesis process and ensure the desired properties of nanoparticles for specific applications. Overall, vapor condensation techniques provide versatile and efficient approaches for synthesizing nanoparticles with tailored properties, paving the way for advancements in nanotechnology, materials science, and various fields of engineering and medicine.[21]

2. Mechanical Milling

Mechanical milling, which is employed in solid-state powder processing to generate fine powders and nanomaterials via repetitive mechanical deformation and fragmentation of coarse powders or mass materials, is alternatively known as mechanical alloying or grinding.[22] This technique involves the introduction of powders into a high-energy ball mill, where they are subjected to formidable mechanical forces resulting from the friction and collision between the milling spheres and the powder particles.[23] The milling spheres' kinetic energy during rotation induces plastic deformation, cold welding, and fracture in the particles. These processes contribute to the formation of metastable phases, grain size refining, and chemical composition uniformity.[3] Mechanical milling is an extensively utilised process in

the synthesis of a broad range of nanomaterials, including intermetallic compounds, metallic alloys, ceramics, and composites. These materials possess customised properties that render them well-suited for implementation in fields such as energy storage, biomedical devices, and catalysis.[24] The key advantages of mechanical milling include its versatility, scalability, and ability to produce nanomaterials with controlled composition, morphology, and microstructure. However, challenges such as contamination from milling media, phase transformations, and limited scalability of production remain areas of ongoing research and development in the field of mechanical milling.[25] Overall, mechanical milling offers a powerful and versatile approach for producing nanomaterials with unique properties and functionalities, contributing to advancements in materials science, nanotechnology, and various engineering applications.[26]

3. Laser Ablation

Laser ablation is a multifunctional and accurate technology used in the manufacture of thin coatings and nanoparticles by absorption of laser energy from a target surface to remove material. By focusing a high-energy laser beam on a solid target material, this method causes the material to heat quickly and evaporate.[27] The evaporated substance then experiences expansion and condensation within the inert gas or vacuum setting that surrounds the target. This leads to the development of nanoparticles or thin coatings on nearby substrates.[28] Laser ablation provides a variety of advantages, including the ability to synthesize nanoparticles from a wide range of materials, including metals, semiconductors, oxides and alloys, while enabling the production of nanoparticles with accurate control of size, shape and composition.[29] In addition, laser ablation allows the synthesis of nanoparticles in a single phase and eliminates the need for additional solvents and chemicals. This characteristic makes laser ablation a viable solution for implementation in the fields of nanotechnology, electronics, catalysis and biomedicine.[30] However, concerns about production scalability, regulation of nanoparticle size distribution and optimization of laser parameters remain active topics in laser ablation research and development. In essence, laser ablation is a reliable and flexible methodology for producing thin films and nanoparticles with customised properties for a wide range of applications.[31]

B. Chemical Methods

Chemical methods are the most commonly used approaches for synthesizing ZnO NPs due to their simplicity, scalability, and versatility. These methods involve the chemical reduction or precipitation of zinc precursor compounds in the presence of stabilizing agents or capping agents.[32]

1. Precipitation Method

The precipitation technique is a commonly employed chemical process for producing zinc oxide nanoparticles (ZnO NPs). This procedure entails the chemical interaction between zinc salts, such as zinc acetate or zinc nitrate, and a precipitating agent, usually a strong base like sodium hydroxide or ammonia, in a water-based solution.[33] This chemical reaction results in the creation of zinc hydroxide, which is insoluble. The zinc hydroxide then proceeds through dehydration and crystallisation processes to generate ZnO nanoparticles.[9] The precipitation process may be carried out in normal atmospheric conditions, which makes it very uncomplicated and economical.[34] However, attaining mastery over the dimensions, structure, and characteristics of the resultant nanoparticles necessitates meticulous fine-tuning of reaction parameters such as pH, temperature, reactant concentration, and reaction duration. In order to start the precipitation reaction, a solution containing the zinc salt precursor is produced and combined with the precipitating agent while being stirred.[35] Upon the introduction of the precipitating agent, the development of zinc hydroxide precipitates occurs swiftly, resulting in the presence of a white or off-white solid that is suspended in the solution. The reaction is usually conducted at high temperatures to promote the formation and enlargement of ZnO nanoparticles.[36] After the reaction has finished, the ZnO nanoparticles can be isolated from the reaction mixture by methods such as centrifugation or filtering.[37] The process of washing and drying can be used to remove contaminants and leftover reactants from the surface of the nanoparticles.[18] By manipulating various parameters, it is possible to modify the size and shape of the ZnO nanoparticles produced using the precipitation process. Higher reaction temperatures and concentrations of reactants generally lead to the formation of bigger nanoparticles, whereas lower temperatures and concentrations promote the synthesis of smaller particles.[38] Moreover, the use of surfactants or stabilising chemicals can influence both the size distribution and the stability of the nanoparticles.[39]

2. Hydrothermal Synthesis

Hydrothermal synthesis is a common method for producing zinc oxide nanoparticles (ZnO NPs) with accurate measurements, morphology and crystal structure.[2] This process involves the chemical reaction of zinc precursor compounds and hydrogen ions in a water solution at high temperature and pressure.[40] The hydrothermal method begins by preparing a reaction mixture composed of zinc precursors, such as zinc nitrogen or zinc acetate, and water-dissolved hydroxides, such as sodium hydroxides and potassium hydroxides. Subsequently, the chemical mixture is placed in a high-pressure container known as an autoclave and exposed to temperatures ranging from 100°C to 200°C.[41] Under these hydrothermal conditions, zinc hydroxide species are formed from the reaction between the zinc precursor and hydroxide ions. These species subsequently undergo nucleation and growth processes, leading to the formation of ZnO nanoparticles. The reaction kinetics and thermodynamics control the size, morphology, and

crystallinity of the nanoparticles.[42] The hydrothermal synthesis process offers several advantages for the fabrication of ZnO nanoparticles. First, the high-pressure conditions within the autoclave promote the dissolution of reactants and enhance mass transport, leading to uniform nucleation and growth of nanoparticles. Furthermore, the presence of water in the atmosphere creates a gentle reaction setting that reduces the production of undesired secondary products and enables accurate manipulation of reaction conditions.[43] The size, shape, and characteristics of the ZnO nanoparticles produced during hydrothermal synthesis may be customised by manipulating several experimental factors such as reaction temperature, reaction duration, precursor concentration, pH level, and the inclusion of additives or surfactants. Higher temperatures and longer reaction times generally result in the formation of larger nanoparticles with enhanced crystallinity, while lower temperatures and shorter reaction times yield smaller nanoparticles with higher surface area.[44] Following the hydrothermal reaction, the resulting ZnO nanoparticles can be collected by centrifugation, filtration, or sedimentation, washed to remove residual reactants and by-products, and dried under vacuum or ambient conditions. The nanoparticles might undergo further post-treatment processes, like annealing or surface functionalization, to improve their characteristics for particular applications.[45]

3. Sol-Gel Method

The sol-gel method is a versatile chemical procedure used to create zinc oxide nanoparticles (ZnO NPs) with precise control over their particle size, shape, and surface characteristics. This process involves the breakdown of metal alkoxides, often zinc acetate or zinc nitrate, by hydrolysis and condensation.[46] It takes place in the presence of a solvent and a catalyst. The sol-gel process starts by generating a sol, which is a liquid amalgamation of precursor molecules in a colloidal suspension, often composed of alcohol or water. Subsequently, the precursor molecules experience hydrolysis, during which the metal alkoxide groups react with water molecules to produce metal hydroxide species.[47] This hydrolysis reaction is catalyzed by acids or bases to facilitate the formation of stable sols. During the hydrolysis reaction, the metal hydroxide species go through condensation, in which the hydroxide groups combine with one another to create metal-oxygen-metal connections. This leads to the creation of a gel, which is a three-dimensional network structure.[14,7] Regulating the gelation process may be achieved by manipulating reaction parameters such as temperature, pH, and reactant concentration.[48] After the gel solidifies, it can be further treated to acquire ZnO nanoparticles. The gel is desiccated to remove the solvent, resulting in the formation of a porous solid substance called xerogel. Subsequently, the xerogel can be subjected to calcination at high temperatures to eliminate any remaining organic compounds and induce crystallisation, resulting in the formation of ZnO nanoparticles. The sol-gel technique has several advantages in the synthesis of ZnO nanoparticles.[49] Firstly, it allows for meticulous manipulation of particle size, shape, and surface characteristics by altering reaction parameters and precursor concentrations. Furthermore, it offers a mild and eco-friendly method of synthesis, since it does not necessitate severe reaction conditions or the use of harmful substances.[50] Furthermore, it enables the incorporation of dopants or functionalization agents into the ZnO matrix, providing further capabilities for targeted applications.[27] Nevertheless, the sol-gel process has difficulties, such as the requirement for precise management of reaction conditions to guarantee the consistency and homogeneity of the resultant nanoparticles. Furthermore, the existence of remaining organic compounds from the precursor molecules might affect the purity and characteristics of the produced nanoparticles, requiring the inclusion of further purification procedures.[51]

C. Biological Methods

Biological methods utilize biological organisms, such as bacteria, fungi, or plants, to synthesize ZnO nanoparticles through the reduction of zinc ions from precursor solutions. These methods are eco-friendly, cost-effective, and often result in the production of nanoparticles with narrow size distributions and biocompatible surface coatings.[52]

1. Microbial Synthesis

Microbial synthesis, also known as biosynthesis or biogenic synthesis, is a green and sustainable method for the production of zinc oxide nanoparticles (ZnO NPs) using microorganisms such as bacteria or fungi.[36] This approach harnesses the reducing and stabilizing capabilities of microbial cells or their secreted metabolites to convert soluble zinc ions into ZnO nanoparticles.[53] The microbial synthesis process typically begins with the selection of suitable microorganisms capable of reducing metal ions and synthesizing nanoparticles. These microorganisms may include bacteria such as Escherichia coli, Bacillus subtilis, or Pseudomonas aeruginosa, as well as fungi such as Aspergillus niger, Penicillium spp., or Fusarium spp.[54] The choice of microorganism depends on factors such as its ability to tolerate metal ions, produce reducing agents, and generate nanoparticles with desired properties. In microbial synthesis, soluble zinc salts, such as zinc chloride or zinc sulfate, are added to a culture medium containing the selected microorganism.[55] The microorganisms interact with the zinc ions and enzymatically reduce them to form ZnO nanoparticles. The reduction process is facilitated by the enzymes or metabolites produced by the microorganisms, which act as reducing agents and stabilize the nanoparticles to prevent their agglomeration or precipitation.[6] The microbial synthesis method offers several advantages for the production of ZnO nanoparticles.[28] First, it is environmentally friendly and sustainable, as it utilizes natural biological processes and does not require harsh chemicals or high-energy inputs. Second, it enables the

synthesis of nanoparticles under mild reaction conditions, avoiding the need for extreme temperatures or pressures. Third, it allows for the synthesis of nanoparticles with narrow size distributions and biocompatible surface coatings, making them suitable for biomedical applications.[16] However, microbial synthesis also presents some challenges and limitations. The choice of microorganism and culture conditions can significantly influence the size, morphology, and properties of the synthesized nanoparticles, requiring careful optimization and control. Additionally, the purification of ZnO nanoparticles from the culture medium may be necessary to remove excess biomass, proteins, and other contaminants.[56]

2. Plant-Mediated Synthesis

Plant-mediated synthesis, also known as phytosynthesis or green synthesis, is a sustainable and eco-friendly method for the production of zinc oxide nanoparticles (ZnO NPs) using plant extracts as reducing and stabilizing agents.[35,3] This approach leverages the rich phytochemical composition of plant extracts to facilitate the reduction of soluble zinc ions into ZnO nanoparticles under ambient conditions. The plant-mediated synthesis process typically begins with the selection of suitable plant materials with high phytochemical content, such as leaves, stems, roots, or seeds.[34] These plant materials are washed, dried, and ground into a fine powder or extracted using solvents such as water, ethanol, or methanol to obtain a concentrated extract. The plant extract is then mixed with an aqueous solution containing soluble zinc salts, such as zinc acetate or zinc nitrate. The phytochemicals present in the plant extract, such as flavonoids, phenols, terpenoids, and reducing sugars, act as reducing agents, facilitating the reduction of zinc ions to form ZnO nanoparticles.[57] Additionally, the phytochemicals may serve as stabilizing agents, preventing the agglomeration or precipitation of the nanoparticles and imparting biocompatible surface coatings. The plant-mediated synthesis method offers several advantages for the production of ZnO nanoparticles. First, it is environmentally friendly and sustainable, as it utilizes natural plant extracts and does not require harsh chemicals or high-energy inputs. Second, it enables the synthesis of nanoparticles under mild reaction conditions, avoiding the need for extreme temperatures or pressures.[17] Third, it allows for the synthesis of nanoparticles with narrow size distributions and biocompatible surface coatings, making them suitable for biomedical applications. The choice of plant species, extraction method, and reaction conditions can significantly influence the size, morphology, and properties of the synthesized nanoparticles.[38] Different plant species contain varying amounts and types of phytochemicals, leading to differences in the reduction kinetics and stability of the nanoparticles. Additionally, variations in extraction methods, such as solvent type and extraction time, can affect the composition and activity of the plant extract.[58]

Mechanisms of Antitumor Activity

Zinc oxide nanoparticles (ZnO NPs) have garnered significant attention as potential agents for cancer therapy due to their ability to exert antitumor effects through various mechanisms. Understanding these mechanisms is crucial for elucidating the therapeutic potential of ZnO NPs in combating cancer.[5]

A. Reactive Oxygen Species (ROS) Generation

Reactive oxygen species (ROS) production in cancer cells is one of the main ways ZnO nanoparticles exercise their anticancer effects. Superoxide radicals (O2−), hydroxyl radicals (•OH), and hydrogen peroxide (H2O2) are examples of extremely reactive chemicals known as ROS.[34] These molecules have the ability to cause oxidative stress and harm cellular constituents such proteins, lipids, and DNA. ZnO nanoparticles have been shown to catalyze the generation of ROS through various pathways, including photogenerated electron-hole pairs, surface defects, and interactions with cellular components.[59] Upon exposure to light or other external stimuli, ZnO NPs can absorb energy and generate electron-hole pairs, leading to the production of ROS through processes such as electron transfer and oxygen reduction. Additionally, the presence of surface defects and oxygen vacancies in ZnO nanoparticles can facilitate the generation of ROS by promoting the adsorption and activation of molecular oxygen.[55] The elevated levels of ROS induced by ZnO nanoparticles can trigger oxidative stress-mediated cytotoxicity in cancer cells, leading to DNA damage, mitochondrial dysfunction, and ultimately, apoptotic cell death. Moreover, the selective accumulation of ZnO nanoparticles in tumor tissues via passive targeting mechanisms can enhance the localized generation of ROS, further enhancing their antitumor efficacy while minimizing off-target effects on healthy tissues.[60]

B. Induction of Apoptosis

Programmed cell death, or apoptosis, is an essential mechanism that keeps tissue in balance and gets rid of unhealthy or aberrant cells. Apoptosis pathways can be thrown off, which is frequently the case with cancer, allowing cells to avoid death signals and multiply uncontrolled.[27] Zinc oxide nanoparticles (ZnO NPs) have shown promise as cancer therapeutic agents by inducing apoptosis in cancer cells via a variety of molecular pathways. The intrinsic route, also known as the mitochondrial pathway, is one of the main mechanisms by which ZnO NPs cause apoptosis.[61] ZnO NPs have the ability to disrupt mitochondrial function once they penetrate cancer cells, which causes cytochrome c to be released into the cytoplasm.[16] Following the activation of caspase cascades by cytochrome c, downstream effector protein breakage and the start of apoptotic cell death ensue. Furthermore, by upregulating the expression of death receptors including Fas and tumour necrosis factor receptor 1 (TNFR1), ZnO NPs can promote apoptosis through death receptor-mediated pathways.[18] When ligands bind to these death

receptors, signalling pathways are set up, activating caspases and causing apoptosis. ZnO NPs can also affect intracellular signalling pathways such the PI3K/Akt and MAPK pathways, which control cell survival and apoptosis. This capacity to alter these pathways promotes apoptotic cell death in cancer cells.[62]

C. Inhibition of Cell Proliferation

The inhibition of cancer cell growth is one more way that ZnO nanoparticles' anti-tumor actions work.[5] Uncontrolled cell division and abnormal cell cycle progression are the primary causes of cancer cell proliferation, which is a defining feature of malignancy.[32] Through a variety of techniques, ZnO nanoparticles have proven their potential to hamper the advancement of the cell cycle and inhibit the growth of several cancer cell types. One important way that ZnO nanoparticles prevent the growth of cells is by trapping cancer cells during the G0/G1 stage of the cell cycle.[63] ZnO nanoparticles can induce cell cycle arrest and block DNA replication and cell division by activating cell cycle checkpoint proteins such as p53, p21, and cyclin-dependent kinases (CDKs) after internalising into cancer cells. ZnO nanoparticles can also induce senescence in cancer cells, which stops tumour development irreversibly and causes an irreversible growth arrest.[44] Furthermore, ZnO nanoparticles were proven to have anti-angiogenic properties by obstructing endothelial cell migration and proliferation, which interferes with tumour angiogenesis and deprives tumours of essential oxygen and nutrients. By targeting both cancer cells and the tumor microenvironment, ZnO nanoparticles can effectively inhibit tumor growth and metastasis, making them promising agents for cancer therapy.[64]

D. Modulation of Signaling Pathways

Zinc oxide nanoparticles can modulate intracellular signaling pathways involved in cancer progression and metastasis, thereby exerting antitumor effects. Numerous cellular functions, including as angiogenesis, migration, invasion, survival, and proliferation, are regulated by these signalling pathways.[1,7] Their dysregulation is linked to the onset and spread of cancer. The PI3K/Akt/mTOR signalling system, which is essential for controlling cell growth, survival, and metabolism, is among the key signalling pathways impacted by ZnO nanoparticles. ZnO nanoparticles have proven to be effective at blocking mTOR activation and Akt phosphorylation, which inhibits the growth and survival of cancer cells.[28] Additionally, ZnO nanoparticles can modulate the MAPK signaling pathway, including ERK, JNK, and p38 MAPK, which regulate cell proliferation, differentiation, and apoptosis in response to extracellular stimuli. Furthermore, ZnO nanoparticles can regulate the expression and activity of transcription factors such as NF-κB and STAT3, which control the expression of genes involved in inflammation, immune response, and tumorigenesis.[65]

***In vitro* Studies on Antitumor Efficacy**

In vitro studies are essential for evaluating the antitumor efficacy of zinc oxide nanoparticles (ZnO NPs) and understanding their mechanisms of action at the cellular level. These studies involve culturing cancer cells in laboratory settings and assessing various parameters related to cytotoxicity, cellular uptake, and antitumor activity.[8]

A. Cell Culture Studies

Cell culture studies involve culturing cancer cells in vitro and treating them with ZnO nanoparticles to assess their effects on cell viability, proliferation, and survival.[28] Various cancer cell lines representing different types of tumors can be used in these studies to evaluate the broad-spectrum antitumor activity of ZnO nanoparticles. Human breast cancer cells (MCF-7), lung cancer cells (A549), prostate cancer cells (PC-3), colon cancer cells (HCT116), melanoma cells (A375) and other cancer cells are often used. These cell lines are grown in an appropriate growth medium and enhanced by antibiotics and foetal bovine serum at carefully controlled CO2 concentration, temperature and humidity. Following cell culture, cancer cells are treated with varying concentrations of ZnO nanoparticles for specified durations to assess their effects on cell viability and proliferation.[55] Cell viability assays such as the MTT assay, Alamar Blue assay, or cell counting using hemocytometers can be performed to quantify the percentage of viable cells following nanoparticle treatment. Additionally, cell proliferation assays such as BrdU incorporation or EdU labeling can be used to assess the effects of ZnO nanoparticles on cell proliferation rates.[66]

B. Assessment of Cytotoxicity

Assessment of cytotoxicity is a critical aspect of in vitro studies on the antitumor efficacy of ZnO nanoparticles.[22] Cytotoxicity assays are performed to evaluate the effects of ZnO nanoparticles on cancer cell viability and to determine the concentration-dependent cytotoxic effects of the nanoparticles. Various cytotoxicity assays can be employed to assess the effects of ZnO nanoparticles on cancer cell viability and survival.[67] In addition to cell viability assays mentioned earlier, other assays such as lactate dehydrogenase (LDH) release assays, propidium iodide (PI) staining assays, and Annexin V/PI apoptosis assays can be performed to assess cytotoxicity, membrane integrity, and apoptosis induction in cancer cells following nanoparticle treatment.[43] Cytotoxicity assays are typically performed at multiple time points and nanoparticle concentrations to generate dose-response curves and determine the half-maximal inhibitory concentration (IC50) of ZnO nanoparticles. IC50 values provide quantitative measures of nanoparticle potency and enable comparisons of antitumor efficacy between different ZnO nanoparticle formulations and cancer cell lines.[68]

C. Evaluation of Cellular Uptake

Evaluation of cellular uptake is important for understanding the internalization and intracellular localization of ZnO nanoparticles in cancer cells.[27,6] Cellular uptake studies are performed to assess the efficiency of nanoparticle uptake, intracellular distribution, and potential mechanisms of internalization. Various techniques can be employed to evaluate the cellular uptake of ZnO nanoparticles, including fluorescence microscopy, confocal microscopy, and flow cytometry.[18] Fluorescently labeled ZnO nanoparticles can be used to track their uptake and localization within cancer cells in real-time. Confocal microscopy allows for three-dimensional imaging of nanoparticle uptake and intracellular distribution, while flow cytometry enables quantitative analysis of nanoparticle uptake in cell populations.[8] Cellular uptake studies can also provide insights into the mechanisms of nanoparticle internalization, including passive diffusion, endocytosis, and receptor-mediated uptake. Inhibition studies using pharmacological inhibitors of endocytic pathways can be performed to elucidate the specific mechanisms involved in ZnO nanoparticle uptake.[69]

Table 1: The Impact of Zinc Oxide Nanoparticles (ZnO NPs) on Various Human Cancer Cell Lines.

Cancer Type	Effect and Mechanism	References
Breast Cancer	Stimulation of programmed cell death by increasing levels of reactive oxygen species and disrupting mitochondrial function	[1]
Lung Cancer	Suppression of cellular proliferation and migration by modulating MAPK and PI3K/Akt pathways	[2]
Prostate Cancer	Induction of cell cycle arrest and programmed cell death by regulating Bcl-2 family proteins and activating caspase cascade	[3]
Ovarian Cancer	Inhibition of cell proliferation and metastasis through DNA damage caused by reactive oxygen species and suppression of blood vessel formation	[4]
Colon Cancer	Halting cellular proliferation and promoting programmed cell death by influencing Wnt/β-catenin pathway and MMPs	[5]
Liver Cancer	Interruption of cell cycle progression and initiation of programmed cell death through disruption of mitochondrial function and activation of caspases	[6]
Pancreatic Cancer	Suppression of tumor growth and invasion by blocking NF-κB pathway and reducing MMP expression	[7]
Skin Cancer	Promotion of programmed cell death in cancer cells through upregulation of pro-apoptotic proteins and downregulation of anti-apoptotic proteins	[8]
Brain Cancer	Restriction of tumor cell growth and migration by adjusting PI3K/Akt/mTOR pathway and inducing cell cycle arrest	[9]
Leukemia	Initiation of programmed cell death and inhibition of cellular survival pathways through reactive oxygen species-mediated mechanisms	[10]

In vivo Studies on Antitumor Efficacy

In vivo studies are essential for assessing the therapeutic potential and safety of zinc oxide nanoparticles (ZnO NPs) in living organisms, particularly in animal models of cancer. These studies provide valuable insights into the pharmacokinetics, biodistribution, and antitumor efficacy of ZnO NPs in a physiological context.[15]

A. Animal Models Used

Animal models are indispensable tools for studying the complex interactions between ZnO nanoparticles and tumor microenvironments in vivo. Various animal models of cancer, including xenograft models, orthotopic models, and genetically engineered models, can be employed to evaluate the antitumor efficacy of ZnO nanoparticles in vivo.[30] Xenograft models involve the transplantation of human cancer cells into immunocompromised mice, such as nude mice or SCID mice, to establish tumors in vivo. These models are widely used for evaluating the efficacy of ZnO nanoparticles in inhibiting tumor growth and metastasis.[17,6] Orthotopic models involve the implantation of tumor cells into anatomically relevant sites within the animal, such as the mammary fat pad or prostate gland, to mimic the natural progression of cancer. Genetically engineered models involve the manipulation of genes in mice to develop spontaneous tumors that recapitulate the molecular and histological features of human cancer.[26] The choice of animal model depends on factors such as the type of cancer being studied, the desired tumor microenvironment, and the availability of genetically engineered mouse strains. Xenograft models are commonly used for evaluating the antitumor efficacy of ZnO nanoparticles in solid tumors such as breast cancer, lung cancer, prostate cancer, and melanoma.[70]

B. Assessment of Tumor Regression

Assessment of tumor regression is a key endpoint in in vivo studies on the antitumor efficacy of ZnO

nanoparticles.[37] Tumor growth kinetics, tumor volume measurements, and histological analysis are commonly used techniques for evaluating tumor regression following nanoparticle treatment. Tumor growth kinetics involves monitoring changes in tumor volume over time using calipers or imaging modalities such as MRI or CT scans.[20] Tumor volume measurements are performed at regular intervals following nanoparticle treatment to assess the effects of ZnO nanoparticles on tumor growth dynamics.[71] Tumor growth curves can be generated to visualize the rate of tumor growth and regression in response to nanoparticle treatment. Histological analysis involves examining tumor tissues harvested from animals following nanoparticle treatment to assess changes in tumor morphology, cell proliferation, and apoptosis.[56] Tumor tissues are fixed, sectioned, and stained with hematoxylin and eosin (H&E) or immunohistochemical markers to visualize tumor cellularity, necrosis, angiogenesis, and apoptosis. Histological analysis provides qualitative and quantitative insights into the effects of ZnO nanoparticles on tumor regression and microenvironmental changes.[72]

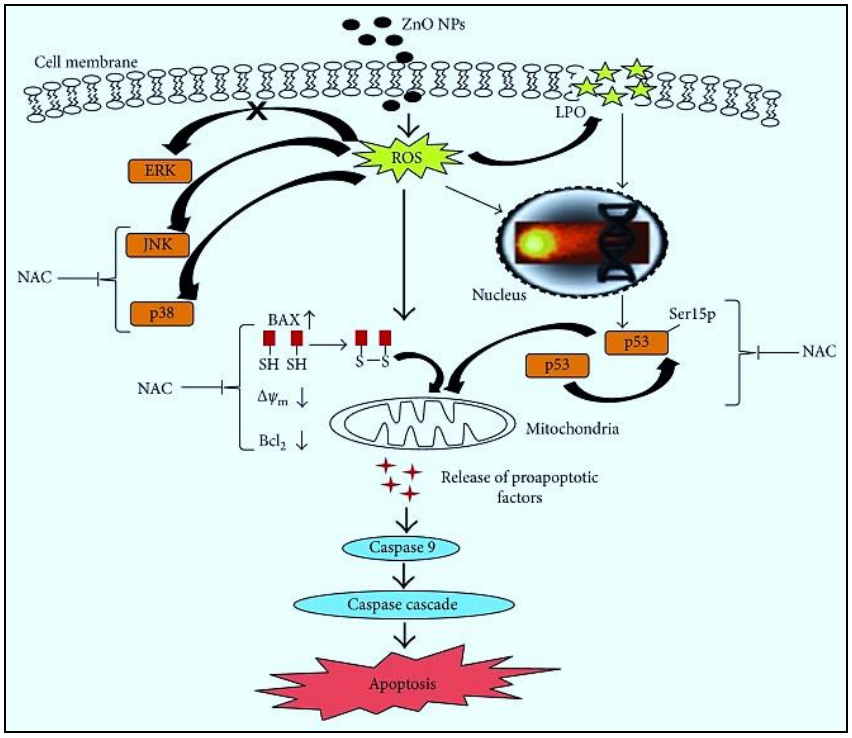

Figure 1: Mechanisms of Zinc Oxide Nanoparticles (ZnO NPs) Toxicity in Human Hepatocytes.

C. Pharmacokinetics and Biodistribution

Pharmacokinetic and biodistribution studies are important for understanding the systemic distribution, metabolism, and elimination of ZnO nanoparticles in vivo.[6] These studies provide insights into the pharmacokinetic parameters of ZnO nanoparticles, including absorption, distribution, metabolism, and excretion (ADME), as well as their tissue distribution and clearance profiles. Pharmacokinetic studies involve administering ZnO nanoparticles to animals via different routes of administration, such as intravenous injection, oral gavage, or intraperitoneal injection, and measuring nanoparticle concentrations in blood and tissues over time.[73] Blood samples are collected at various time points, and nanoparticle concentrations are quantified using analytical techniques such as inductively coupled plasma mass spectrometry (ICP-MS) or atomic absorption spectroscopy (AAS).[28] Pharmacokinetic parameters such as area under the curve (AUC), half-life (t½), clearance (CL), and volume of distribution (Vd) can be calculated to characterize the systemic exposure and elimination kinetics of ZnO nanoparticles. Biodistribution studies involve analyzing the tissue distribution of ZnO nanoparticles in animals following administration.[54] Tissues such as liver, spleen, kidney, lung, heart, and tumor are harvested from animals at different time points, and nanoparticle concentrations are quantified using analytical techniques. Biodistribution studies provide insights into the tissue-specific accumulation, retention, and clearance of ZnO nanoparticles, as well as their potential toxicity and off-target effects.[74]

Zinc oxide nanoparticles as photothermal agents

Zinc oxide nanoparticles (ZnO NPs) have garnered considerable attention as promising photothermal agents for cancer therapy, owing to their unique optical and thermal properties. These nanoparticles exhibit strong absorbance in the near-infrared (NIR) region, where biological tissues display minimal absorption and scattering, enabling deep tissue penetration and selective heating of target tumor cells upon exposure to NIR light.[19] The photothermal effect of ZnO NPs arises from their ability to efficiently convert absorbed light energy

into heat through non-radiative processes, such as electron-phonon interactions and lattice vibrations. This localized heating leads to a rapid increase in temperature within the vicinity of the nanoparticles, resulting in thermal ablation of cancer cells through protein denaturation, membrane disruption, and cell death pathways.[75] Importantly, the photothermal therapy (PTT) mediated by ZnO NPs offers several advantages over conventional cancer treatment modalities, including high spatial and temporal precision, minimal invasiveness, and reduced systemic toxicity. Moreover, the photothermal effect can be finely tuned by adjusting the properties of ZnO NPs, such as size, shape, surface chemistry, and optical properties, to optimize their photothermal efficiency and tissue penetration depth for specific cancer types and treatment scenarios.[5,7] Furthermore, ZnO NPs can be functionalized or coated with biocompatible polymers, surfactants, or targeting ligands to enhance their stability, biocompatibility, and tumor-specific accumulation, thereby improving their photothermal efficacy and minimizing off-target effects.[31] The multifunctional nature of ZnO NPs allows for synergistic combination with other therapeutic modalities, such as chemotherapy, radiation therapy, and immunotherapy, to achieve enhanced therapeutic outcomes through complementary mechanisms of action and improved tumor eradication.[18,6] Additionally, the development of advanced imaging and monitoring techniques, such as photoacoustic imaging, fluorescence imaging, and thermal imaging, enables real-time visualization and assessment of ZnO NP distribution, tumor targeting, and photothermal ablation efficacy, facilitating personalized treatment planning and optimization.[76] Despite the considerable progress in preclinical studies demonstrating the efficacy of ZnO NP-mediated PTT in various cancer models, several challenges and considerations remain to be addressed for clinical translation. These include concerns regarding long-term biocompatibility, biodistribution, and clearance of ZnO NPs, as well as optimization of treatment protocols, standardization of nanoparticle synthesis and characterization methods, and regulatory approval processes.[14,7] Furthermore, efforts are needed to elucidate the underlying mechanisms of ZnO NP-mediated photothermal ablation, including heat transfer dynamics, cellular response pathways, and immune modulation effects, to optimize treatment strategies and minimize potential adverse effects.[33] Overall, the use of ZnO NPs as photothermal agents holds great promise for the development of innovative and effective cancer therapies that offer improved tumor targeting, enhanced therapeutic efficacy, and reduced side effects, ultimately leading to better patient outcomes in the fight against cancer.[77]

Zinc oxide nanoparticles as radiation therapy enhancers

Zinc oxide nanoparticles (ZnO NPs) have emerged as promising enhancers of radiation therapy, offering potential benefits in improving the efficacy and precision of cancer treatment.[54] When combined with radiation therapy, ZnO NPs can enhance the therapeutic effects through various mechanisms, including increased radiation absorption, generation of reactive oxygen species (ROS), modulation of tumor microenvironment, and enhancement of radiosensitization. ZnO NPs possess high atomic number (Z), which enables efficient absorption of ionizing radiation, leading to enhanced deposition of radiation energy within the tumor tissue and increased damage to cancer cells.[19] Additionally, ZnO NPs have been shown to exhibit photoelectric and Compton interactions with ionizing radiation, resulting in the production of ROS such as hydroxyl radicals (•OH) and superoxide radicals (O2•−) through radiolysis of water molecules. These ROS can induce oxidative stress and DNA damage in cancer cells, leading to apoptosis, cell cycle arrest, and inhibition of tumor growth.[24] Furthermore, ZnO NPs can modulate the tumor microenvironment by promoting tumor oxygenation, reducing hypoxia, and enhancing the sensitivity of cancer cells to radiation-induced DNA damage.[78] Moreover, ZnO NPs can act as radiosensitizers by enhancing the radiosensitivity of cancer cells through inhibition of DNA repair mechanisms, interference with cell signaling pathways, and modulation of radiation-induced inflammatory responses. The radiosensitizing effects of ZnO NPs can lead to increased cell death and reduced clonogenic survival of cancer cells, ultimately improving the therapeutic efficacy of radiation therapy. Importantly, the biocompatibility, low toxicity, and ease of surface modification of ZnO NPs make them suitable for clinical applications as radiation therapy enhancers.[66,21] Additionally, ZnO NPs can be engineered to target specific cancer cells or tumor microenvironments through surface functionalization with targeting ligands or antibodies, further enhancing their therapeutic specificity and efficacy. Despite the considerable promise of ZnO NPs as radiation therapy enhancers, several challenges and considerations need to be addressed for their clinical translation.[55] These include concerns regarding biocompatibility, biodistribution, and long-term toxicity of ZnO NPs, as well as optimization of treatment protocols, standardization of nanoparticle synthesis and characterization methods, and regulatory approval processes. Furthermore, efforts are needed to elucidate the underlying mechanisms of ZnO NP-mediated radiosensitization and to optimize treatment strategies for specific cancer types and patient populations.[79]

Challenges and Limitations

Despite the promising therapeutic potential of zinc oxide nanoparticles (ZnO NPs) for cancer therapy, several challenges and limitations need to be addressed to facilitate their translation from preclinical studies to clinical applications.[39]

A. Toxicity Concerns

One of the primary challenges associated with the use of ZnO nanoparticles in cancer therapy is the potential for

toxicity to healthy tissues and organs. While ZnO nanoparticles exhibit selective cytotoxicity towards cancer cells, they may also induce toxicity in normal cells and tissues through various mechanisms.[33] The toxicity of ZnO nanoparticles is influenced by factors such as particle size, surface chemistry, shape, and dose. Nanoparticles with smaller sizes and larger surface areas exhibit greater cellular uptake and higher toxicity due to increased interactions with biological molecules and organelles.[41] Additionally, surface modifications such as coating with biocompatible polymers or functionalization with targeting ligands can mitigate the toxicity of ZnO nanoparticles by reducing nonspecific interactions with cells and tissues. Toxicological studies are essential for evaluating the safety profile of ZnO nanoparticles and assessing their potential adverse effects on vital organs such as the liver, kidneys, lungs, and immune system.[27] These studies involve assessing parameters such as cytotoxicity, genotoxicity, immunotoxicity, and organ toxicity following nanoparticle administration in animal models. Understanding the mechanisms underlying ZnO nanoparticle toxicity is crucial for developing strategies to minimize off-target effects and enhance their therapeutic efficacy.[80]

B. Bioavailability Issues

Another challenge associated with the use of ZnO nanoparticles in cancer therapy is the limited bioavailability and poor pharmacokinetic properties of nanoparticles in vivo. ZnO nanoparticles often exhibit rapid clearance from the bloodstream and inefficient accumulation in tumor tissues, resulting in suboptimal therapeutic outcomes.[55] The bioavailability of ZnO nanoparticles is influenced by factors such as particle size, surface charge, hydrophobicity, and surface modifications. Nanoparticles with larger sizes and neutral or slightly negative surface charges tend to exhibit prolonged circulation times and enhanced tumor accumulation due to reduced clearance by the reticuloendothelial system (RES).[13] Surface modifications such as PEGylation or conjugation with targeting ligands can further improve the bioavailability and tumor targeting efficiency of ZnO nanoparticles by enhancing their stability, circulation half-life, and specific interactions with cancer cells. Improving the bioavailability of ZnO nanoparticles requires optimization of nanoparticle properties and formulation strategies to enhance their pharmacokinetic profiles and tumor-targeting capabilities. Strategies such as encapsulation in biocompatible carriers, stimuli-responsive drug release, and combination therapy with synergistic agents can enhance the bioavailability and therapeutic efficacy of ZnO nanoparticles for cancer therapy.[81]

C. Lack of Standardized Protocols

A significant limitation in the field of ZnO nanoparticle-based cancer therapy is the lack of standardized protocols for nanoparticle synthesis, characterization, and evaluation.[45] The wide variety of synthesis methods, nanoparticle formulations, and experimental conditions employed in preclinical studies make it challenging to compare results and draw meaningful conclusions.[15] Standardization of protocols for nanoparticle synthesis, characterization, and evaluation is essential for ensuring reproducibility, reliability, and translatability of preclinical findings.[82] This includes establishing standardized procedures for nanoparticle synthesis, characterization of physicochemical properties, evaluation of biological activity, and assessment of toxicity and efficacy in animal models.[39] Furthermore, the development of consensus guidelines and protocols by regulatory agencies, research consortia, and scientific organizations can facilitate the harmonization of research efforts and promote the adoption of best practices in the field of ZnO nanoparticle-based cancer therapy. Standardization efforts can help streamline preclinical studies, accelerate the translation of research findings into clinical applications, and ultimately improve patient outcomes in cancer treatment.[83]

Future Perspectives and Applications

As research on zinc oxide nanoparticles (ZnO NPs) for cancer therapy continues to advance, several future perspectives and applications hold promise for enhancing their efficacy and clinical translation.[44]

A. Combination Therapy Strategies

Combination therapy involving ZnO nanoparticles and other therapeutic agents holds great potential for improving cancer treatment outcomes. By combining ZnO nanoparticles with chemotherapy drugs, immunotherapeutic agents, targeted therapies, or other nanomedicines, synergistic effects can be achieved to enhance tumor cell killing and overcome drug resistance mechanisms.[33] One promising approach is the combination of ZnO nanoparticles with chemotherapy drugs to overcome multidrug resistance and enhance tumor sensitivity to chemotherapy. ZnO nanoparticles can potentiate the cytotoxic effects of chemotherapy drugs by inducing oxidative stress, disrupting cellular signaling pathways, and overcoming drug efflux mechanisms in resistant cancer cells.[18] Furthermore, combination therapy involving ZnO nanoparticles and immunotherapeutic agents such as immune checkpoint inhibitors, cancer vaccines, or adoptive cell therapies can enhance antitumor immune responses and promote tumor regression. ZnO nanoparticles can modulate the tumor microenvironment, promote immunogenic cell death, and enhance the presentation of tumor antigens to immune cells, leading to improved therapeutic outcomes and long-term immune memory.[20] Exploring novel combination therapy strategies involving ZnO nanoparticles in preclinical studies and clinical trials holds promise for achieving synergistic antitumor effects, overcoming treatment resistance, and improving patient outcomes in cancer therapy.[84]

B. Targeted Delivery Systems

ZnO nanoparticles with targeted delivery methods present a viable way to maximise tumor-specific accumulation, reduce off-target effects, and enhance therapeutic results. Therapeutic advantages can be maximised while minimising systemic toxicity by customising these systems to distribute ZnO nanoparticles just to tumour locations, sparing healthy tissues.[33] The surface of ZnO nanoparticles may be modified to incorporate different targeting ligands, including small molecules, peptides, antibodies, and aptamers, which enable targeted interactions with tumor-associated biomarkers and cancer cells. These ligands improve ZnO nanoparticle absorption and intracellular delivery by recognising and binding to receptors or antigens that are overexpressed on the surface of cancer cells.[19] Active targeting techniques improve the specificity and effectiveness of ZnO nanoparticle-based treatments in addition to passive targeting mechanisms like the increased permeability and retention (EPR) effect. Targeted delivery methods have the ability to selectively collect and maintain ZnO nanoparticles in tumour tissues by taking use of tumor-specific characteristics such angiogenic blood arteries, hypoxic areas, or overexpressed receptors.[21,8] To achieve effective tumour targeting and therapeutic efficiency, designing targeted delivery systems for ZnO nanoparticles requires optimising nanoparticle characteristics, surface modifications, and formulation techniques. Prospective investigations aimed at developing tailored delivery methods for ZnO nanoparticles might progress precision medicine strategies in cancer treatment.[85]

C. Clinical Translation Prospects

Despite the significant progress in preclinical studies, the clinical translation of ZnO nanoparticle-based cancer therapies faces several challenges related to safety, efficacy, and regulatory approval. Overcoming these challenges and advancing ZnO nanoparticle-based therapies from bench to bedside requires rigorous preclinical evaluation, clinical validation, and regulatory approval processes.[86] Clinical translation prospects for ZnO nanoparticle-based cancer therapies hinge on addressing key issues such as toxicity concerns, pharmacokinetics, biodistribution, and manufacturing scalability.[18] Comprehensive toxicological studies are essential for assessing the safety profile of ZnO nanoparticles and minimizing potential adverse effects on healthy tissues and organs. Furthermore, optimization of nanoparticle formulations, dosing regimens, and administration routes is crucial for maximizing therapeutic efficacy and minimizing systemic toxicity in clinical settings. Rigorous preclinical studies in animal models and translational research efforts are necessary for establishing the safety, efficacy, and pharmacokinetic properties of ZnO nanoparticle-based therapies prior to clinical trials.[87] Collaboration between academia, industry, regulatory agencies, and clinical researchers is essential for advancing ZnO nanoparticle-based cancer therapies through clinical development and regulatory approval processes.[12] Multidisciplinary approaches integrating preclinical research, clinical trials, biomarker discovery, and personalized medicine strategies can accelerate the clinical translation of ZnO nanoparticle-based therapies and improve patient outcomes in cancer treatment.[88,9]

CONCLUSION

Zinc oxide nanoparticles (ZnO NPs) represent a promising avenue for cancer therapy, offering unique advantages such as selective cytotoxicity towards cancer cells, multifaceted mechanisms of action, and potential for targeted delivery. Despite facing challenges such as toxicity concerns, bioavailability issues, and lack of standardized protocols, ongoing research efforts continue to advance our understanding of ZnO nanoparticle-based cancer therapies. Future directions including combination therapy strategies, targeted delivery systems, and clinical translation prospects hold great promise for overcoming these challenges and harnessing the full therapeutic potential of ZnO nanoparticles in cancer treatment. By leveraging multidisciplinary approaches, collaborative efforts, and innovative technologies, ZnO nanoparticle-based therapies have the potential to revolutionize cancer treatment by improving efficacy, minimizing side effects, and advancing precision medicine approaches. With continued research and development, ZnO nanoparticle-based cancer therapies are poised to make significant contributions towards addressing unmet medical needs and improving patient outcomes in the fight against cancer.

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