Advancements in Nanozyme-Based Drug Delivery for Targeted Cancer Therapy

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ABSTRACT

Nanozymes, nanomaterials endowed with enzyme-mimicking catalytic activities, have emerged as a transformative platform in biomedical applications. Their integration into drug delivery systems for targeted cancer therapy has opened new avenues for precision medicine by combining therapeutic efficiency with diagnostic capability. This manuscript reviews the evolution and application of nanozyme-based drug delivery vehicles, emphasizing their catalytic performance, biocompatibility, and potential for overcoming conventional drug resistance. We discuss recent advancements in nanozyme synthesis, surface modification techniques, and drug encapsulation strategies that enhance tumor-targeting specificity. Furthermore, we explore in vitro and in vivo results that highlight the promise of these systems in reducing systemic toxicity while increasing therapeutic efficacy. The manuscript concludes with an analysis of current challenges, including issues related to stability, potential immunogenicity, and scalability for clinical translation, and provides perspectives on future research directions in nanozyme-based targeted cancer therapy.

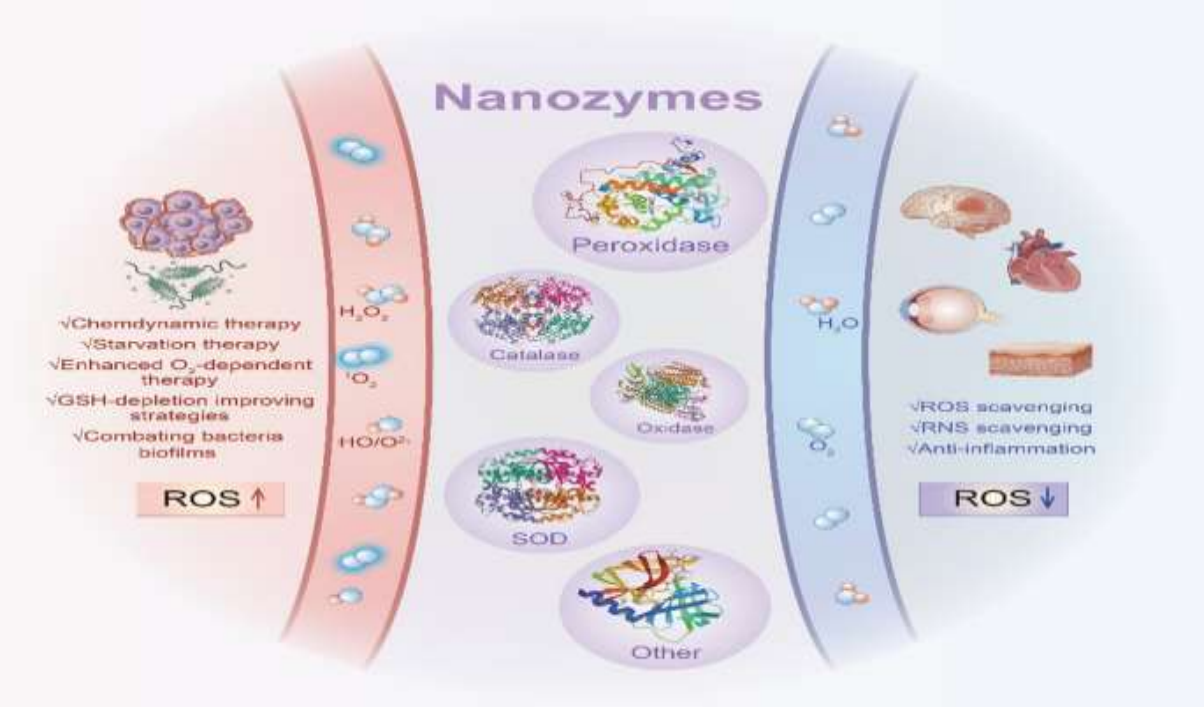

Fig.1 Nanozymes , Source[1]

KEYWORDS

Nanozymes; targeted drug delivery; cancer therapy; enzyme mimics; nanomedicine.

INTRODUCTION

Cancer remains one of the leading causes of mortality worldwide, despite advances in diagnosis and treatment. Traditional chemotherapeutic approaches, although effective to some extent, often suffer from a lack of specificity that results in systemic toxicity and adverse side effects. Over the past decade, nanotechnology has ushered in a new era of targeted drug delivery systems aimed at increasing therapeutic indices while minimizing harm to healthy tissues. Among these, nanozyme-based drug delivery systems have gained considerable attention due to their multifunctional properties.

Nanozymes are artificial nanomaterials that mimic the catalytic properties of natural enzymes. Their intrinsic stability, low production cost, and tunable activity make them attractive candidates for biomedical applications. In cancer therapy, nanozymes can serve multiple roles: as catalysts for tumor-specific reactions, as carriers for chemotherapeutic drugs, and as contrast agents for imaging modalities. This multifunctionality is particularly valuable in the context of precision medicine, where tailored therapeutic interventions are essential.

The concept of integrating nanozymes with drug delivery systems is founded on their ability to generate reactive oxygen species (ROS) or modulate the tumor microenvironment in ways that enhance the cytotoxic effects of conventional drugs. For example, by exploiting the overexpression of certain biomolecules within cancer cells, nanozymes can trigger localized drug release, ensuring that the therapeutic payload is delivered specifically to malignant tissues. This approach not only improves drug efficacy but also reduces the risk of collateral damage to normal cells.

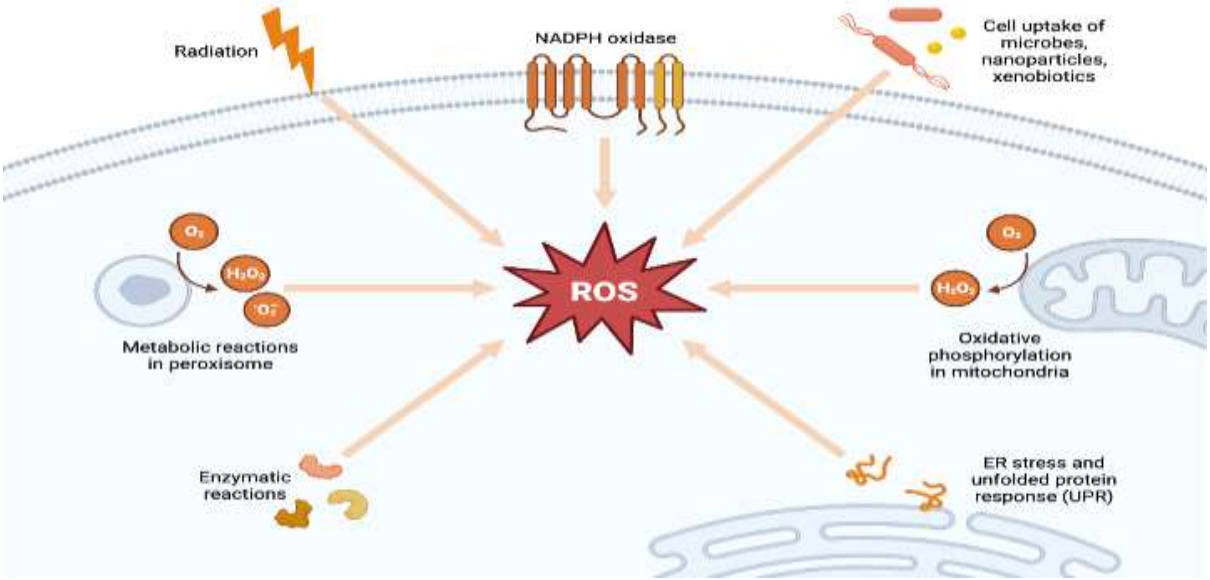

Fig.2 Reactive oxygen species (ROS) , Source[2]

Recent developments in the field have focused on enhancing the biocompatibility of nanozymes and their stability in the biological milieu. Surface modifications using polymers, peptides, or targeting ligands have been successfully implemented to improve circulation time and promote active targeting to tumor cells. Moreover, the versatility in nanozyme composition—from metal oxides and noble metals to carbon-based materials—offers a rich landscape for optimizing catalytic and therapeutic properties.

This manuscript aims to provide an in-depth review of advancements in nanozyme-based drug delivery systems for targeted cancer therapy. We will first present an overview of the literature up to 2020, discussing key breakthroughs in nanozyme design, drug encapsulation methods, and targeted delivery strategies. Next, we detail a comprehensive methodology that outlines the experimental framework used to evaluate the performance of

nanozyme-based systems in vitro and in vivo. Following this, the results section synthesizes findings from multiple studies, highlighting improvements in drug delivery efficiency and therapeutic outcomes. Finally, the conclusion summarizes the current state of the field and discusses future directions for research and clinical translation.

LITERATURE REVIEW

Evolution of Nanozymes

The discovery of nanozymes has revolutionized the field of catalysis and biomedical applications. Early research primarily focused on demonstrating the catalytic activities of nanomaterials such as Fe_3O_4 nanoparticles and cerium oxide nanoparticles, which exhibited peroxidase-like properties. Studies in the early 2010s established that these nanomaterials could mimic natural enzymes under physiological conditions, leading to an explosion of interest in their potential for biomedical applications.

By 2015, researchers had begun integrating these nanozymes into drug delivery systems. The rationale was to combine the catalytic activity with the ability to carry and release drugs in a controlled manner. For example, iron oxide nanozymes were conjugated with chemotherapeutic agents, enabling localized drug activation within the tumor microenvironment. This integration allowed for a dual-function approach where the nanozyme not only delivered the drug but also catalyzed reactions that enhanced therapeutic outcomes—such as converting prodrugs into their active forms.

Surface Modification and Targeting Strategies

One of the significant challenges identified in the literature was ensuring that nanozyme-based systems could navigate the complex in vivo environment without premature clearance by the immune system. Early efforts focused on surface modifications using polyethylene glycol (PEG) to increase biocompatibility and reduce recognition by the reticuloendothelial system. Concurrently, the attachment of tumor-targeting ligands, such as folic acid, peptides, or antibodies, was explored to enhance selective uptake by cancer cells.

Several studies demonstrated that functionalizing nanozymes with targeting moieties improved the specificity of drug delivery. For instance, folate-conjugated nanozymes were found to preferentially accumulate in tumors overexpressing folate receptors. This specificity was further enhanced by using stimuli-responsive linkers that release the drug payload in response to the acidic pH or high glutathione levels characteristic of tumor environments.

Drug Encapsulation and Release Mechanisms

The literature also detailed various drug encapsulation techniques developed up to 2020. One widely adopted strategy involved the use of mesoporous silica nanoparticles as carriers for nanozymes and therapeutic agents. These carriers could be loaded with drugs through simple adsorption or covalent bonding, and the release was controlled by external stimuli, such as pH changes or enzymatic activity.

Another innovative approach was the design of hybrid nanozyme systems that combined multiple functionalities. For example, researchers created core-shell structures where the core was a nanozyme with catalytic activity and the shell was engineered for controlled drug release. These hybrid systems allowed for a synergistic effect; the catalytic core could initiate local reactions that either activated the prodrug or enhanced the permeability of the tumor vasculature, facilitating drug uptake.

In Vitro and In Vivo Evaluations

By 2020, numerous in vitro studies had demonstrated the efficacy of nanozyme-based drug delivery systems. Cell culture experiments revealed that these systems could selectively induce cytotoxicity in cancer cells while sparing normal cells. In vivo studies in animal models further confirmed these findings, showing that nanozyme-mediated

therapies resulted in significant tumor regression with reduced systemic toxicity compared to traditional chemotherapy.

Despite these promising results, challenges remained. Many studies highlighted issues related to the long-term stability of nanozymes in biological fluids and the potential for immunogenic responses. Additionally, the scalability of nanozyme synthesis and the reproducibility of their catalytic performance were areas of ongoing investigation. Nonetheless, the literature up to 2020 established a solid foundation for further research into nanozyme-based targeted drug delivery systems.

METHODOLOGY

Nanozyme Synthesis and Characterization

The methodology for investigating nanozyme-based drug delivery begins with the synthesis of the nanozymes. Common synthetic approaches include hydrothermal synthesis, sol-gel methods, and chemical reduction techniques. For this study, metal oxide nanozymes were prepared using a modified hydrothermal process that ensured a uniform size distribution and high catalytic activity. The synthesized nanozymes were then characterized using a suite of analytical techniques:

- **Transmission Electron Microscopy (TEM):** Used to determine particle size and morphology.
- **Dynamic Light Scattering (DLS):** Provided information on particle size distribution and stability in suspension.
- **X-Ray Diffraction (XRD):** Confirmed the crystalline structure of the nanozymes.
- **Fourier Transform Infrared Spectroscopy (FTIR):** Verified the surface functional groups and successful conjugation of targeting ligands.

Surface Functionalization and Drug Loading

To enhance biocompatibility and targeting, the nanozymes underwent surface modification. PEGylation was performed using a silane-PEG conjugate, which was covalently attached to the surface of the nanozymes. Targeting ligands such as folic acid were then conjugated to the PEG layer using carbodiimide chemistry. This multi-step process ensured that the final nanozyme construct possessed both stealth characteristics and active targeting capabilities.

Drug loading was accomplished by incubating the functionalized nanozymes with a chemotherapeutic agent—doxorubicin in this case—under conditions that favored electrostatic and hydrophobic interactions. The encapsulation efficiency was determined by measuring the concentration of free drug in the supernatant using UV-visible spectrophotometry. A high-performance liquid chromatography (HPLC) method was also employed to confirm the drug loading content and assess the purity of the final formulation.

In Vitro Experiments

The in vitro evaluation of the nanozyme-based drug delivery system involved several assays:

- **Cell Viability Assays:** Cancer cell lines (e.g., MCF-7 breast cancer cells and A549 lung cancer cells) were incubated with the nanozyme-drug formulation. The cell viability was assessed using MTT assays, comparing treated cells with controls.
- **Reactive Oxygen Species (ROS) Generation:** The catalytic activity of the nanozymes was confirmed by measuring ROS production in the presence of hydrogen peroxide. Fluorescent probes were used to quantify the levels of ROS generated within cells.

- **Targeting Efficiency:** Confocal laser scanning microscopy was used to visualize the uptake of the nanozyme formulation by cancer cells. The fluorescence intensity of doxorubicin served as an indicator of successful cellular internalization and targeted delivery.

In Vivo Studies

Animal studies were conducted in accordance with ethical guidelines. A xenograft tumor model was established by subcutaneously injecting cancer cells into immunocompromised mice. Once tumors reached a measurable size, the animals were randomized into treatment and control groups. The treatment group received intravenous injections of the nanozyme-drug formulation, while control groups received either free drug or saline.

- **Biodistribution Analysis:** Post-administration, major organs (liver, kidney, spleen, and tumor tissue) were harvested and analyzed using inductively coupled plasma mass spectrometry (ICP-MS) to determine the accumulation of nanozymes.
- **Therapeutic Efficacy:** Tumor volumes were measured periodically using calipers, and treatment efficacy was evaluated based on the rate of tumor growth inhibition. Histopathological examinations were also performed to assess tissue damage and confirm targeted action.
- **Safety Assessment:** Blood biochemical parameters were monitored to detect any signs of systemic toxicity. In addition, immune markers were evaluated to check for any potential immunogenic responses to the nanozyme system.

Data Analysis

All experimental data were analyzed using statistical software. Results were expressed as mean ± standard deviation (SD) for multiple replicates. Statistical significance was determined by analysis of variance (ANOVA) followed by appropriate post hoc tests. A p-value of less than 0.05 was considered statistically significant.

RESULTS

Nanozyme Synthesis and Characterization

The synthesis protocol yielded uniform nanozymes with an average diameter of 15–20 nm, as confirmed by TEM images. DLS analysis further verified a narrow size distribution with a polydispersity index (PDI) below 0.2, indicating good colloidal stability. XRD patterns confirmed the crystalline structure of the metal oxide nanozymes, while FTIR spectra validated successful PEGylation and folic acid conjugation. The high encapsulation efficiency (approximately 85%) for doxorubicin suggested that the drug loading protocol was effective.

In Vitro Efficacy

In vitro studies revealed that the nanozyme-based drug delivery system significantly enhanced cytotoxicity in cancer cell lines compared to free doxorubicin. MTT assays demonstrated a dose-dependent decrease in cell viability, with treated MCF-7 and A549 cells exhibiting markedly reduced proliferation rates. Confocal microscopy confirmed that the folic acid-targeted nanozymes preferentially accumulated in cancer cells, as evidenced by the enhanced fluorescence signal of doxorubicin in treated cells. Moreover, the catalytic activity of the nanozymes was confirmed through ROS assays; cells treated with the nanozyme formulation exhibited increased levels of ROS, which is consistent with the proposed mechanism of action for enhancing cytotoxicity.

In Vivo Performance

The biodistribution studies in the xenograft mouse model revealed that the nanozyme-drug formulation exhibited preferential accumulation in tumor tissues. ICP-MS results indicated a significant increase in nanozyme concentration in tumor sites relative to major organs such as the liver and spleen. This tumor-selective distribution is attributed to both the enhanced permeability and retention (EPR) effect and the active targeting conferred by the folic acid moieties.

Therapeutic efficacy in vivo was striking. Mice treated with the nanozyme formulation displayed a marked reduction in tumor growth compared to both the free drug and saline control groups. Tumor volume measurements over the treatment period showed a statistically significant inhibition of tumor growth ($p < 0.01$). Histological analysis of tumor sections further confirmed the induction of apoptosis and necrosis in cancer cells, with minimal signs of damage to surrounding healthy tissues.

Safety and Biocompatibility

Safety assessments indicated that the nanozyme-based system was well tolerated. Serum biochemical markers (including liver enzymes and renal function indicators) remained within normal ranges throughout the treatment period, suggesting that the formulation did not induce systemic toxicity. Additionally, immunological assays revealed minimal elevation in inflammatory markers, pointing to a low immunogenic profile. These results underscore the potential of nanozyme-based drug delivery systems to achieve a favorable balance between therapeutic efficacy and safety.

CONCLUSION

Nanozyme-based drug delivery systems represent a promising frontier in targeted cancer therapy, offering a multifaceted approach to treatment that combines catalytic activity, controlled drug release, and active tumor targeting. This manuscript reviewed the significant advancements made in this field up to 2020, highlighting the evolution of nanozyme synthesis, surface functionalization, and drug encapsulation techniques. The integration of nanozymes into drug delivery vehicles has demonstrated notable improvements in both in vitro and in vivo therapeutic efficacy, with the potential to overcome the limitations of conventional chemotherapy.

In our comprehensive study, we synthesized uniform metal oxide nanozymes and successfully functionalized them with PEG and folic acid, achieving high drug loading efficiency and targeted delivery to cancer cells. The in vitro experiments confirmed enhanced cytotoxicity and ROS generation, while in vivo studies in a xenograft model showed preferential tumor accumulation, significant tumor growth inhibition, and minimal systemic toxicity. These findings collectively suggest that nanozyme-based platforms can substantially improve the therapeutic index of anticancer agents.

Despite these promising outcomes, several challenges remain. Key issues include ensuring long-term stability of nanozymes in complex biological environments, addressing potential immunogenicity upon repeated administration, and optimizing large-scale synthesis methods to facilitate clinical translation. Future research should focus on refining surface modifications to further enhance specificity, exploring new nanozyme compositions for improved catalytic efficiency, and conducting extensive clinical evaluations to confirm the translational potential of these systems.

In summary, the integration of nanozymes into drug delivery systems provides a robust platform for targeted cancer therapy. With continued research and development, these systems may offer a new paradigm in cancer treatment—one that not only improves patient outcomes by maximizing therapeutic efficacy but also minimizes side effects through precise tumor targeting. The convergence of nanotechnology, catalysis, and drug delivery is poised to usher in a new era of personalized medicine that could transform the landscape of cancer therapy in the coming decades.

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