

Multimodal Therapies for Pancreatic Ductal Adenocarcinoma: Ultrasound-Stimulated Nanoconstructs based on Lipid-coated Zinc Oxide

Summary

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive and lethal forms of cancer, expected to become the second leading cause of cancer-related deaths worldwide by 2030. Despite extensive research, PDAC remains challenging to treat due to its dense and desmoplastic tumor microenvironment (TME), high chemoresistance, and complex biological pathways. The TME is characterized by hypoxia and immunosuppression, which severely limit the efficacy of conventional therapies and hinder the infiltration of therapeutic agents. Consequently, there is an urgent need for innovative treatment strategies that can overcome these inherent challenges and improve patient outcomes.

This PhD dissertation proposes a nanoengineered therapeutic approach based on the remote activation of lipid-coated, iron-doped zinc oxide nanoparticles (ZnO NPs) using safe ultrasound (US) stimulation. The research aims to address the limitations of existing therapies by developing a customizable and multifunctional nanoplatform capable of delivering enhanced therapeutic outcomes while minimizing systemic toxicity. The dissertation is structured into three key chapters that progressively build the foundation and application of this innovative treatment strategy.

The first chapter provides a comprehensive overview of PDAC pathophysiology, exploring tumor genesis, progression, and the intricacies of the TME that contribute to the disease's resistance to standard treatments. This section reviews current therapeutic options, including chemotherapy, radiation therapy, and emerging multimodal and theranostic approaches that combine diagnostics with targeted treatment. Special emphasis is given to the potential of nanoparticle-based therapies, which offer unique advantages in terms of precision targeting, controlled release, and the possibility of functional enhancements. The literature review underscores the limitations of existing treatments and establishes the rationale for using nanotechnology to develop more effective, personalized therapies for PDAC.

In the second chapter, the focus shifts to the development and characterization of a tailored lipidic formulation designed as a smart coating for the ZnO NPs. Inspired by COVID-19 vaccine technology, this lipidic shell provides several key functionalities, including enhanced stability, improved biocompatibility, and a platform for further customization. The lipidic coating is optimized to ensure efficient shielding of the nanoparticles, while also enabling the attachment of targeting agents,

peptides, antibodies, or fluorescent molecules for advanced diagnostic and therapeutic applications. Physicochemical characterizations, including stability studies and particle size distribution analyses, confirm the reproducibility and robustness of the nanoconstructs. In vitro experiments on human pancreatic cancer cell lines reveal significant improvements in cytocompatibility and cellular uptake, demonstrating the potential for enhanced therapeutic performance when combined with targeted functionalization.

The third chapter explores the integration of ultrasound stimulation as a non-invasive external trigger to activate the therapeutic potential of the nanoparticles. This combined approach leverages the generation of reactive oxygen species (ROS) under ultrasound exposure, leading to oxidative stress and subsequent cancer cell death. The addition of a sonosensitizer (IR780) to the lipidic formulation further enhances the ROS production, improving the overall antitumor efficacy. In vitro studies using a murine pancreatic cancer cell line demonstrate significant reductions in cell viability when treated with the sonosensitizer-enhanced ZnO NPs and ultrasound. Encouraged by these promising results, an in vivo study is conducted on subcutaneous murine PDAC models. The nanoconstructs are administered intratumorally to maximize local bioavailability and minimize systemic off-target effects. Biodistribution studies reveal efficient tumor retention of the nanoparticles and minimal accumulation in non-target organs, highlighting their potential safety profile.

The combined therapy of nanoparticles and ultrasound stimulation results in substantial antitumor effects, including tumor shrinkage, increased immune cell infiltration, and prolonged survival in treated animals. Flow cytometry and histological analyses indicate significant macrophage polarization, increased immune cell activity and apoptosis of cancer cells, suggesting that the therapy may stimulate an immune response in addition to its direct cytotoxic effects. Importantly, the individual components of the therapy are inherently safe, with the lipid-coated nanoparticles demonstrating high biocompatibility and the ultrasound stimulation utilizing clinically approved transducers. This ensures a high degree of safety and flexibility for potential clinical applications.

The results presented in this dissertation highlight the potential of combining nanomedicine with physical stimuli to address the complex challenges of PDAC treatment. The customizable nature of the lipidic shell allows for further integration with immunotherapeutic strategies, such as the inclusion of agonistic antibodies or immune-modulating peptides, offering opportunities for synergistic multimodal treatments. Future directions include refining nanoparticle dosages, optimizing administration routes, and exploring additional functional enhancements to maximize therapeutic efficacy and clinical translation.

In conclusion, this dissertation highlights the critical need for highly customizable and personalized approaches to PDAC treatment, combining the latest advancements in nanomedicine with established and novel therapeutic modalities. The dissertation underscores the potential of ultrasound-activated,

lipid-coated ZnO nanoparticles as a powerful tool in the fight against PDAC, paving the way for future research and clinical advancements in multimodal cancer therapy.

