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研究課題名	Ferritin nanocaged doxorubicin for targeted hepatocellular carcinoma therapy	
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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Project Completion)

Project Completion Report
Report
Research Background and Objectives From April 2022 to March 2025, this project was conducted in collaboration with the Institute of Medical Science, the University of Tokyo (IMSUT), under the IMSUT International Joint Usage/Research Center Program. The primary objective was to develop advanced ferritin-based nanomedicines for targeted therapy of esophageal squamous cell carcinoma (ESCC), a highly prevalent and deadly cancer with limited treatment options. Despite the clinical success of platinum-based chemotherapy, its application in ESCC is hindered by drug resistance, systemic toxicity, and poor patient outcomes. Our research aimed to overcome these challenges through a two-pronged strategy: designing structure-guided ferritin-platinum (Pt) prodrugs for enhanced drug delivery and integrating PD-L1 blockade with T-cell activation for improved immunotherapy. The first approach focused on leveraging the natural nanocage structure of ferritin for precise drug encapsulation and targeted delivery. However, we discovered that conventional cisplatin (CDDP) is inactivated when encapsulated within ferritin due to interactions with thiol-containing amino acids on the protein surface. To address this, we adopted a structure-guided design strategy, engineering Pt(IV) prodrugs with modified ligands to prevent this deactivation and enhance therapeutic efficacy. The second approach aimed to overcome the limited response of ESCC to PD-L1 blockade by developing a multifunctional nanomedicine platform (ITFn-Pt(IV)) that combined PD-L1 inhibition, chemotherapy, and T-cell activation. This system was designed to not only deliver chemotherapeutic agents but also activate antitumor immunity, providing a synergistic therapeutic effect. Research Progress and Achievements Throughout the three-year project period, we achieved substantial progress, which led to the publication of two high-impact research articles. The first paper, published in ACS Nano (2024), demonstrated the successful design of ferritin-based Pt(IV) prodrugs. Structural analysis revealed that the deactivation of cisplatin within ferritin nanocages was primarily due to its interaction with thiol groups on the protein surface, particularly cysteine residues. Guided by these findings, we synthesized six Pt(IV) prodrugs with modified axial ligands, ensuring that they retained their activity even after encapsulation within ferritin. Among these, the HFn-Pt(IV)-3 variant exhibited the highest antitumor activity, significantly surpassing free cisplatin in both cisplatin-sensitive and cisplatin-resistant ESCC cells. In animal models, including patient-derived xenografts (PDX), HFn-Pt(IV)-3 demonstrated

nstrated superior tumor inhibition with reduced systemic toxicity, highlighting the potential of structure-guided nanomedicine design.

The second publication, appearing in *Advanced Healthcare Materials* (2023), focused on addressing the limited efficacy of PD-L1 blockade in ESCC due to poor T-cell infiltration. We developed an innovative immunotherapy platform, ITFn-Pt(IV), that integrated three therapeutic mechanisms: PD-L1 blockade, chemotherapy, and T-cell activation. This system utilized a genetically engineered ferritin nanocage displaying PD-L1-targeting nanobodies (KN035) and T-cell-activating peptides (Thymosin α 1), while encapsulating Pt(IV) prodrugs. The ITFn-Pt(IV) system demonstrated superior antitumor activity in ESCC models, significantly enhancing T-cell infiltration and tumor regression. Unlike conventional PD-L1 blockade, which has a low response rate in ESCC, this platform effectively activated T-cells and induced immunogenic cell death in tumor cells, providing a synergistic effect.

Both publications acknowledged the support of the IMSUT International Joint Usage/Research Center Program, underlining the importance of this international collaboration. Our research outcomes not only advanced the understanding of ferritin-based drug delivery but also demonstrated the potential of multifunctional nanomedicines in cancer therapy.

International Collaboration and Exchange Activities

International collaboration was a critical component of this project. From January 14 to 17, 2025, project members, including Professors Xiyun Yan, Bing Jiang, Liziang Gao, and Kelong Fan, visited the Institute of Medical Science, the University of Tokyo (IMSUT), Japan. During this visit, the team met with Professor Kawaguchi and his research group, gaining valuable insights into advanced nanomedicine strategies. The team also toured Professor Kawaguchi's laboratory, where they observed cutting-edge research techniques. Professor Kelong Fan delivered an invited lecture at IMSUT, presenting our research findings and engaging in in-depth discussions with Japanese researchers. This visit not only strengthened our collaboration but also fostered knowledge exchange, enhancing the quality and impact of our research.

The collaborative nature of this project extended beyond direct visits. Regular online meetings were held to discuss research progress, share experimental data, and troubleshoot technical challenges. The support from IMSUT facilitated access to advanced research facilities and provided a platform for critical feedback on our research strategy.

Research Significance and Impact

This project has made significant contributions to the field of nanomedicine and cancer therapy. The structure-guided design of ferritin-based Pt(IV) prodrugs demonstrated how a deep understanding of molecular interactions can transform drug delivery strategies, enhancing both efficacy and safety. The development of the ITFn-Pt(IV) platform introduced a novel approach to combining immunotherapy and chemotherapy, addressing the critical challenge of immune evasion in ESCC. Our findings provide a strong foundation for the future development of multifunctional nanomedicines

for cancer treatment.

Scientific Impact: Our work has been published in two high-impact journals, ACS Nano and Advanced Healthcare Materials, highlighting the novelty and importance of our research.

Clinical Potential: The ITFn-Pt(IV) system represents a promising candidate for clinical translation, offering a dual strategy of direct tumor cytotoxicity and immune activation.

International Collaboration: The partnership with IMSUT not only supported the research but also enriched our team's understanding of medical applications.

Future Directions

Building on this project, we plan to expand our research in the following directions:

Further optimization of the ferritin-Pt(IV) prodrug system for other tumor types, including those with multidrug resistance.

Continued collaboration with IMSUT to explore new applications of ferritin-based nanomedicines and strengthen our international research network.

This project has laid a strong foundation for future advancements in tumor immunotherapy, and we look forward to further exploring the therapeutic potential of nanomedicines in cancer treatment.

Publications:

1. Jiang, B., Chen, X., Wang, S., Wang, S., Ma, S., Lu, Y., Ma, L., Liang, Q., Xiao, H., Zhang, L., Yan, X., & Fan, K. (2024). Structure-Guided Design of Ferritin-Platinum Prodrugs for Targeted Therapy of Esophageal Squamous Cell Carcinoma. ACS Nano, 18(17), 11217–11233. <https://doi.org/10.1021/acsnano.4c00212>
2. Xin, Q., Wang, D., Wang, S., Zhang, L., Liang, Q., Yan, X., Fan, K., & Jiang, B. (2023). Tackling Esophageal Squamous Cell Carcinoma with ITFn-Pt(IV): A Novel Fusion of PD-L1 Blockade, Chemotherapy, and T-cell Activation. Advanced Healthcare Materials, 12(14), 2303623. <https://doi.org/10.1002/adhm.202303623>
3. <https://doi.org/10.1002/adhm.202303623>