胰臟癌的個人化免疫治療平台

Personized Cancer Immunotherapy for Pancreatic Tumor

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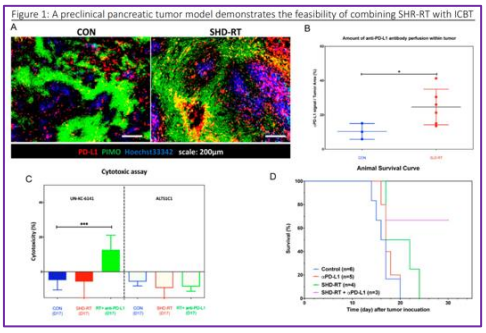
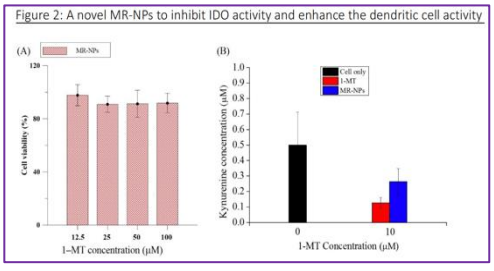
This project aimed to develop a personalized immunotherapeutic platform for treating pancreatic cancer. To achieve this goal, five subproject groups were included. They were (1) to establish a preclinical pancreatic model for testing the efficacy of immunotherapy and monitoring the response to the therapy; to develop (2) an immune modulating therapeutic nanoparticle, (3) a novel self-assembled biomembrane-camouflaged magnetic nanodroplet (SABN), and (4) a cytokine-based immune modulator for cancer therapy; (5) to develop a chip to capture circulating tumor cells for monitoring cancer progression.

During these two years, the first sub-project group has successfully established an orthotopically pancreatic tumor in immune competent mice and demonstrated the feasibility of using a defined radiation therapy protocol to turn an immune unresponsive tumor microenvironment (TME) into one responsive to anti-PD-L1 immunotherapy. Figure 1A demonstrates that the TME was altered following SHD-RT treatment. The SHD-RT-altered TME has better perfusion for ant-PD-L1 antibody delivery (Fig. 1B). Following SHD-RT combined with anti-PD-L1 immune checkpoint blockade therapy (ICBT), mice developed specific cytotoxicity against UN-KC-1647 pancreatic tumor cells, but not ALTS1C1 glioma tumor cells (Fig. 1C), which resulted in 60% tumor free survival (Fig. 1D). This result has been published in IJMS, 2021.

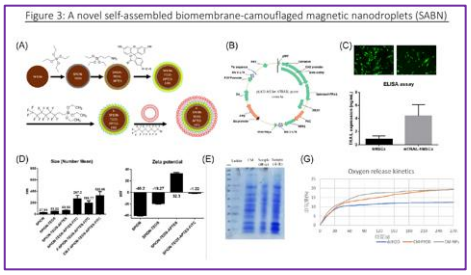


The sub-project group 2 has developed 1-Methyl-tryptophan (1-MT)/R837-loaded nanoparticles (MR-NPs) to inhibit the indoleamine 2,3-dioxygenase (IDO) activity and enhance the dendritic cell activity. Figure 2A shows that the MR-NPs exhibit non-cytotoxic nature toward macrophages even at the high 1-MT concentrations. Figure 2B shows the reduction in kynurenine concentration in the group treated with either free 1-MT or MR-NPs, indicating that the MR-NPs can inhibit the IDO activity. The data indicate that the activity of regulatory T cells could be downregulated with a low kynurenine concentration along with the enhanced antitumor immune response.

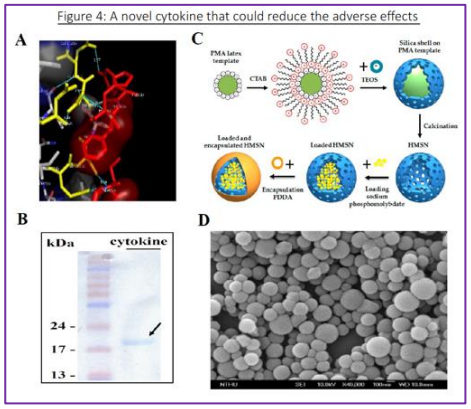


The sub-project 3 group has developed a novel self-assembled biomembrane-camouflaged magnetic nanodroplets (SABN) that could elicit effective apoptosis on human cancer cells. The

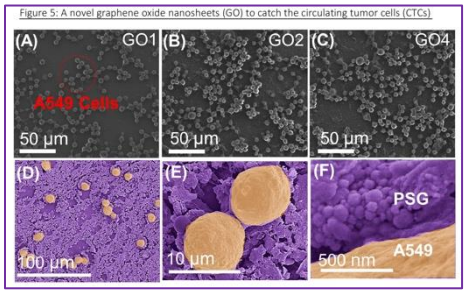
SABN was successfully prepared by integrating the human mesenchymal stem cell (hMSC) membrane with the fluorinated superparamagnetic iron oxide nanoparticles (SPIO) via an efficient self-assembly process (Fig. 3A). Biomembrane with surface-bound TRAIL was harvested from the permanently transfected hMSC (^{mTRAIL}hMSC) which could trigger apoptosis on human cancer cells (Fig. 3B, C). A five-step process was established to prepare the SABN. The evolution of size and zeta potential of the nanoparticle during the synthesis was confirmed using a ZetaSizer (Fig. 3D). Importantly, the preservation of total membrane proteins on SABN was confirmed by SDS-PAGE (Fig. 3E). In addition to its anti-cancer effect, SABN is capable of delivering oxygen which could aid in cancer photodynamic therapy in the future.



The sub-project 4 has developed a novel cytokine that could reduce the adverse effects. The crucial residues of cytokine (Fig. 4A, red color) interact with its receptor (Fig. 4A, yellow color) were modified and recombinant pure cytokine were obtained from protein expression and purification systems (Fig. 4B). Additionally, in order to deliver active form of novel cytokine into targeted place, Prof. Yin collaborated with Prof. Hu of sub-project 5 to perform mesoporous silica nanoparticles (MSN) for protein protection and delivery. Figure 4C indicates the synthesis of hollow mesoporous silica nanoparticles (HMSN), loading with sodium phosphomolybdate and deposition of an external PDDA layer. Figure 4D shows the SEM of vesicles of protein mixture.



Sub-project 5 has developed a graphene oxide nanosheets (GO) to catch the circulating tumor cells (CTCs) by using the surface modification and spin coating approach to have graphene adsorbed on the surface of conductive substrate (indium tin oxide, ITO). This study demonstrated that the thickness and roughness of GO on ITO could be modified by varying binder concentrations. Our result (Fig. 5) shows that the capturing efficiency could be improved from 86% to 96% within 4 minutes by increased roughness (from GO1 to GO4). Furthermore, the resistances of conductive substrates can also reflect to the adsorption of cell numbers via the excellent conductive properties. In the future, we will graft the antibody on the conductive substrate to improve the selectivity of cancer cell capture and increase specificity and also plan to design a higher-throughput capture microfluidic system for drug testing and precision medicine.



Now, we are looking for new funding source to further evaluate the safety and tolerability of novel MR-NPs, SABN, cytokine coated MSN, and GO using our preclinical pancreatic model.