Hepatocellular carcinoma (HCC) is a deadly type of cancer that leads to a mortality rate worldwide. Previous research and pharmaceutical efforts have highlighted the potential of immunotherapy in treating HCC. Immunotherapy enhances or rectify the immune system’s ability to precisely kill the tumor cells. One of the most prominent cell-adoptable immunotherapies is Chimeric Antigen Receptor (CAR) T cell, which has clinically cured some hematopoietic cancers. However, the nature of T cells renders the therapy ineffective against solid tumors with an immunosuppressive tumor microenvironment (TME).

Attempts to overcome the challenge include the co-delivery of immune checkpoint blockers, such as programmed cell death protein 1 (PD-1) antibodies. Among the immunocytes armory, macrophages are equipped with a natural tumor-killing ability, either by phagocytosis or cytokine release. Due to the macrophages’ ability to remodel and penetrate the extracellular matrix, they are also the most persistent immunocytes in solid tumors. Their antigen-presenting ability can cause epipope spread and the recruitment of effector T cells, making them ideal platforms for cell therapy. However, while tumor-associated macrophages (TAMs) may persist and influence the TME, TAMs also express the PD-1 receptor and are subjected to less effective phenotype change.

The research develops a novel design of CAR structure that targets HCC and secretes PD-1 antibody fragments. The CAR structure is introduced into extended pluripotent stem cells (EPSCs) through the PiggyBac transposon to engineer macrophages. Phagocytosis assay, phenotype change assay and peripheral blood mononuclear cells (PBMCs) exhaustion-preventing assay were performed to assess the effectiveness of such CAR macrophages. It is hoped that the EPSC-derived novel CAR macrophage will harbor stronger anti-tumor activities.

Introduction:

1. Hepatocellular carcinoma: Hepatocellular carcinoma (HCC) is a significant health problem worldwide (Luo et al., 2022). Current treatment options for liver cancer include surgery, radiation, targeted therapy and so on (Bui et al., 2023). Phosphatidylinositol-3-(4,5)-bisphosphate-proteoglycan 3 (GPC3) is a key regulator of cell growth and differentiation (Amer et al., 2022). Its expression in tumor tissues highlights it as a potential therapeutic target.

2. Chimeric Antigen Receptor (CAR)

Technique: CAR is a newly developed fusion protein that can be expressed on the surface of T cells. CAR T cells showed profound effectiveness in treating leukemia, lymphoma, and myeloma (June & Sadelain, 2018). However, traditional CAR-T therapy has several limitations, restricting its safety profile and application in solid tumors (Zhang et al., 2023). In view of the unmet needs, one approach is to upgrade CAR constructs to adapt to the safety concerns and solid tumor challenges, see Figure 1.

Figure 1. Current generations of CAR construct. 1st generation: ectodomain of single-chain variable fragment (scFv), hinge, stimulatory module. 2nd generation: one costimulatory module added. 3rd generation adds two costimulatory modules added. 4th generation: cytokine expression domain added.

Methods and Results:

1. CAR-Macrophage: Compared to T cells, macrophages have some natural advantages in immunotherapy.
   - Professional antigen presenting cells to induce epipope spread
   - Active transportation to tumor sites
   - Penetrate into solid tumor via extracellular matrix modelling
   - High persistence within TME
   - Improved safety profile

2. EPSC: Extended pluripotent stem cells (EPSCs) are a more stable cell line with an engineering stability. Indeed, the two major populations of macrophage have distinct differentiation. All stages are induced by the indicated medium. Time points are specified.

3. CAR-Macrophage tumor specificity assay

4. CAR-Macrophage differential affinity assay

Figure 3. CAR expression in engineered human EPSCs and differentiation into monocytes. a. Representative Flow cytometry analysis plots of CAR transfected single colonies. The upper left panel shows the DAPI population (alive) with a signal threshold of 103. The upper right and lower right panels are PBMC coculture and monocytes culture. The upper panel was captured under 10X objective and the lower panel 20X. The red frames highlights translucent cavities.

Figure 4. PBMC exhaustion abusing assay. a. Representative Flow cytometry analysis plots of LAG3 expression in the exhaustion preventing assay. The upper left panel shows the control group resulted from the PBMC exhaustion model. The upper right panel shows the experiment group with 2.5x diluted medium, while the lower right 5x. The LAG3 expression was reduced the most in the 5x diluted condition. b. Representative Flow cytometry analysis plots of PD1 expression in the exhaustion preventing assay. The arrangement of panels follows that of 4b. The PD1 expression was reduced the most in the 2.5x diluted condition.

Future Directions:

1. Macrophage CAR expression assay
2. CAR Macrophage differential affinity assay
3. Macrophage phagocytosis assay
4. CAR Macrophage tumor specificity assay

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Reference:

Bai, J., Huang, M., Song, B., Luo, W., & Ding, R. (2023). The Current Status and Future Prospects for Conversion Therapy in t...