A Single-cell Platform to Investigate the Metabolism of NK and Cancer Cells in CAR-NK Therapy

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Background of research

Recently, chimeric antigen receptor (CAR)-modified immune cell therapy has emerged as a promising treatment for human cancers. The CAR structures expressed by immune cells are designed to specifically target cancer cells by recognizing tumor-associated antigens (TAAs). CAR-T cell therapy was the first cellular immunotherapy applied in clinical translation and commercialization (1). Until now, six CAR-T products have been approved by the U.S. Food and Drug Administration (FDA), and it has achieved considerable success in treating hematological malignancies (2). However, CAR-T therapy still faces hurdles in treating solid tumors. For example, the source of the T cells needs to be autologous (from the same individual), which lengthens the manufacturing time, increases the costs, and restricts the eligibility. In addition, the risks of CAR-T-related cytokine-release syndrome (CRS) and graft-versus-host disease (GvHD) limit the clinical applications of CAR-T therapy (3). Natural killer (NK) cells recognize targets in a human leukocyte antigen (HLA)-unrestricted manner and thus do not carry the GvHD limitations of T cells, making them an attractive candidate for CAR cellular immunotherapy (4). Meanwhile, NK cells have the characteristic of short retention time in the body, which minimizes the toxic side effects of CRS. **Therefore, CAR-NK cellular therapeutics have emerged as a promising next-generation CAR platform with ideal safety and robust therapeutic potential.** Notably, the allogeneic option endows CAR-NK with good availability and low costs. Our preliminary work has successfully established CAR-NK cells targeting the sialylation-modified cancer-derived IgG (SIA-cIgG), which is a novel TAA. CAR-NK targeting SIA-cIgG showed great activities in treating epithelial cancers such as prostate and lung cancers (Fig. 1 and Fig. 2).

Intratumoral heterogeneity is one of the primary factors that impede the efficiency of CAR-NK therapy in solid tumors (5). Metabolic heterogeneity influences the functions of both cancer cells and immune cells. For example, highly glycolytic cancer cells can not only redirect glucose to anabolic reactions in support of proliferation but also secrete an increased amount of lactate, which exerts immunosuppressive effects on T and NK cells (6). Under normal circumstances, NK cells predominantly rely on glucose as their primary metabolic substrate. They use heightened glucose-driven glycolysis and mitochondrial oxidative phosphorylation (OxPhos) to fuel their anti-tumor response (7). However, as described above, tumor cell metabolism deprives metabolic nutrients such

as glucose and glutamine and produces waste products including lactate and hypoxic. It has been reported that the function of NK cells is hindered in the tumor microenvironment (TME) due to metabolic dysfunction (8-10). **Thus, to solve this problem, it is crucial to understand whether/how NK cells adapt to the metabolic suppression of TME.** The existing ways to improve CAR-NK metabolism and anti-tumor activity include mitochondria transfer, activation of the Nrf2 antioxidant pathway, and cytokine engineering (10-12). However, these methods mostly come from the cellular population-level analysis, ignoring the extensive heterogeneity at the genomic, transcriptomic, and proteomic levels within single cells. Population-level analysis inevitably masks the unique information of many individual cells, leading to the loss of crucial insights. **To comprehensively explore the heterogeneity of NK and cancer cells, single-cell analysis is of the prominent value.**

The challenges of single-cell analysis are the isolation, capture, stimulation, and detection of individual cells (13). In recent years, microfluidic technologies have been widely used in single-cell analysis research, with droplet-based microfluidics being a prominent example. These technologies offer advantages such as miniaturization, low cost, high sensitivity, high specificity, and high throughput. They have become powerful tools for the manipulation and analysis of single cells. In droplet-based microfluidic devices, cells are encapsulated within droplets that range from microliter to nanoliter volumes. This allows the analysis of genomic, transcriptomic, proteomic, or metabolomic information from single cells (14). Our collaborator, Professor Xiaoyu Zhou's research group, has developed a single-cell encapsulation system based on a droplet-based microfluidic platform and hydrogel materials. This system utilizes the unique sol-gel transition property of hydrogels, where the hydrogel polymerization is triggered by acidic metabolites generated from cellular metabolism, resulting in the formation of a single-cell encapsulation system (Fig. 3). The platform avoids the need for cell labeling and external manipulation that can affect the cells in traditional methods. Simultaneously, **this single-cell platform can control the hydrogel polymerization based on the accumulation level of acidic metabolites, allowing for the selection of cells with different metabolic profiles for subsequent analysis**.

Thus, in this project, we will use the single-cell platform to monitor the metabolic changes of cancer cells and NK cells in CAR-NK cell therapy. By analyzing the correlation between cellular metabolism and NK cell cytotoxicity, we can reveal the properties of NK cells with good anti-tumor activity in the presence of different cancer cells. **The results can help guide the enrichment of active NK cells for clinical therapy based on tumor heterogeneity.**

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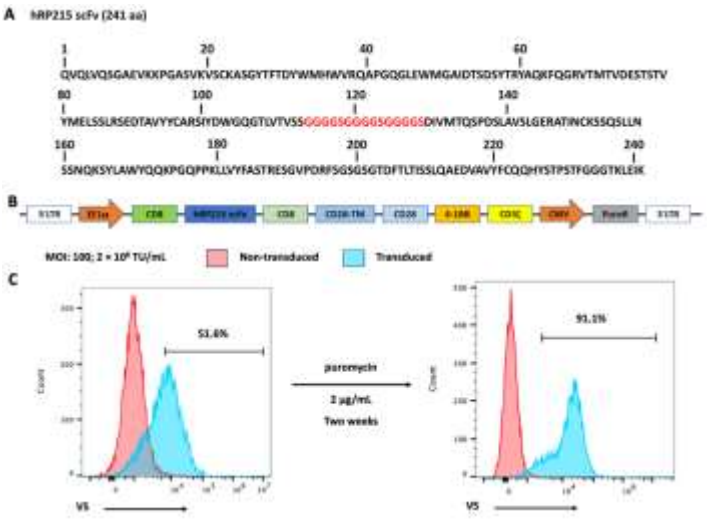

Fig. 1. Construction of anti-SIA-cIgG CAR-NK92 cells. (A) The scFV sequence of RP215 was cloned in to the CAR construct (B) compatible with lentivirus packaging. Recombinant lentivirus was used to transduce the CAR construct to NK92 cell to generate anti-SIA-cIgG CAR-NK92 cells (transduction efficiency = 51.6%). After selection by puromycin, the anti-SIA-cIgG CAR-positive ratio was increased from 51.6% to 91.1%.

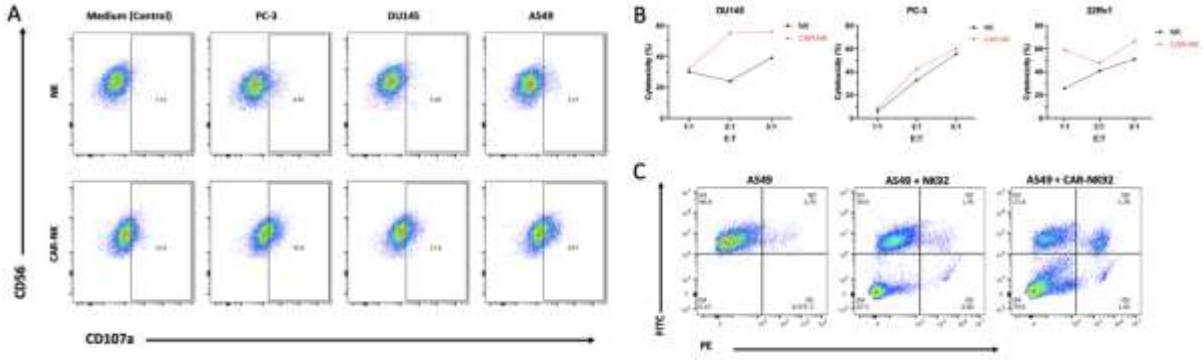

Fig. 2. In vitro anti-cancer activity of SIA-cIgG CAR-NK92 cells. (A) activation of SIA-cIgG CAR-NK92 by prostate cancer and lung cancer cells. (B-C) Killing of indicated cancer cells by SIA-cIgG CAR-NK92 cells. We then evaluate the activity of SIA-cIgG CAR-NK92 cells in killing cancer cells.

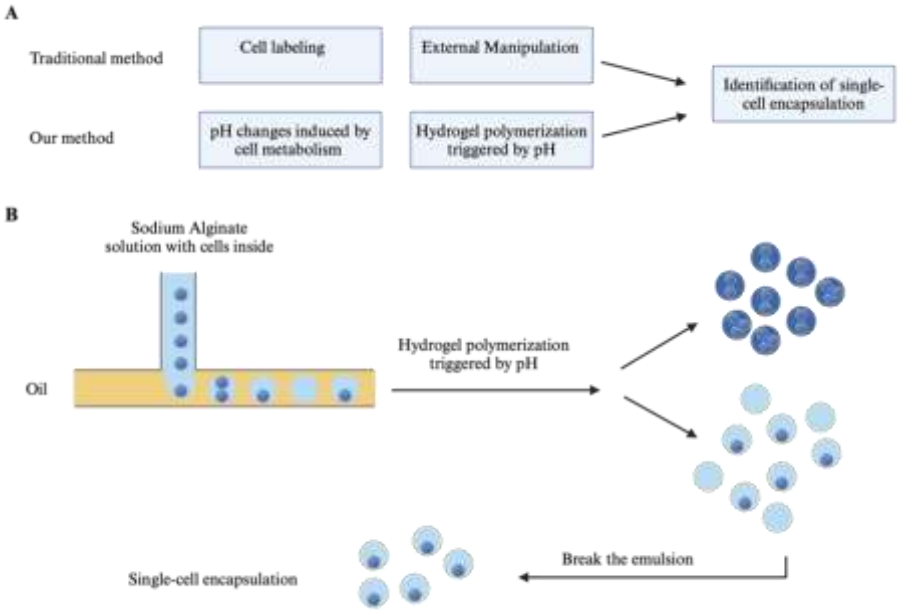

Fig. 3. The diagram showing the single-cell encapsulation method used in this project (A) Comparison with the traditional method. (B) Schematic diagram of the experimental principle.

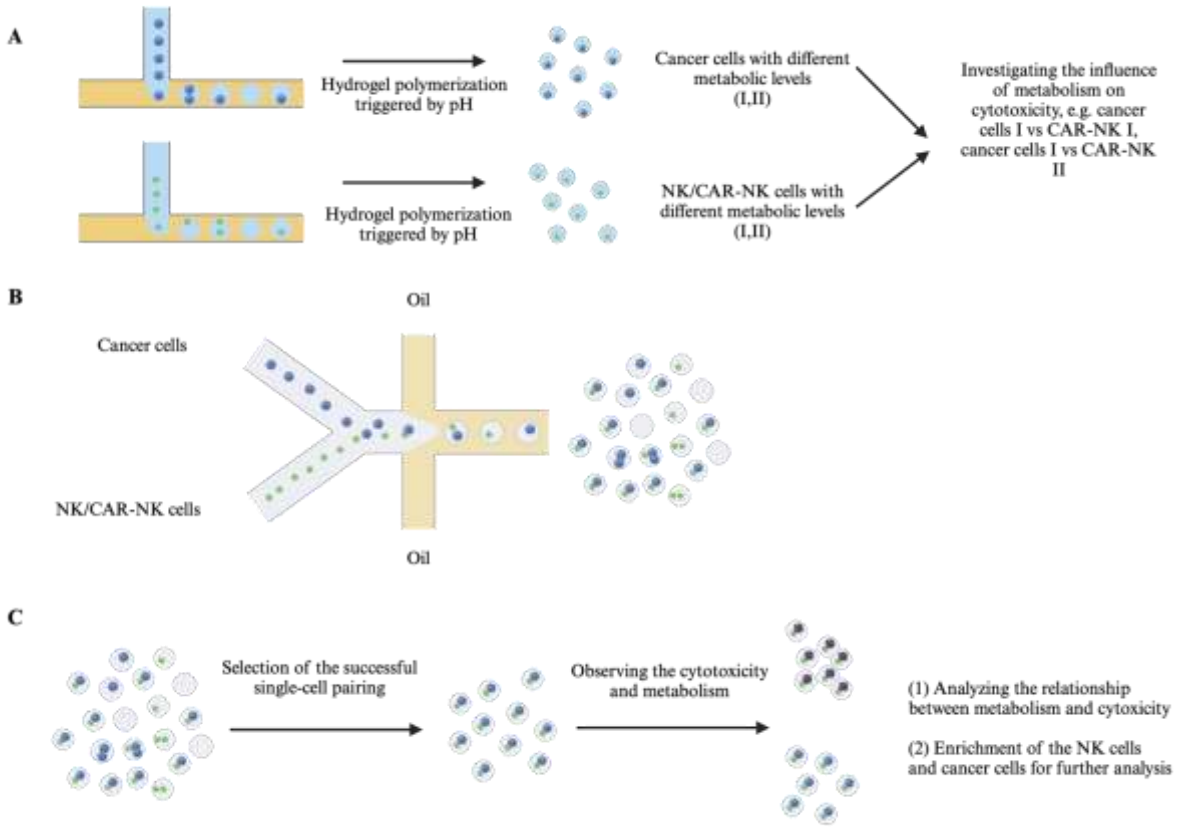

Fig. 4. The diagram showing the research plan. (A) Investigate the metabolism of NK and cancer cells via the hydrogel single-cell encapsulation system. (B) Establish the high-throughput single-cell pairing and coculture system. (C) Monitor the metabolism of NK and cancer cell pairing and investigate the correlation between metabolism and NK cytotoxicity.