Assessing on-target cytotoxicity of standardized CAR T cells co-culture model using gastrointestinal organoids

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BACKGROUND

Developing investigational ex vivo models that accurately replicate the intricate interactions between tissues and immune cells is crucial for advancing novel immunotherapeutic approaches. However, such co-culture systems are often challenging to establish and limited in their scope. Here, we present a new method that makes use of healthy organoids to investigate the interactions between gastrointestinal (GI) epithelium and CAR-T cells, as well as to assess any potential cytotoxic effects of CAR-T cells on the organoids. Our co-culture system uses the Gri3D® platform to generate single GI organoids to which immune cells can be added. The interactions between the organoids and the immune cells are visualized using cell tracker dyes, allowing us to monitor the co-culture evolution over multiple days. Moreover, we utilize live imaging and image analysis techniques to quantify the cytotoxic effects and dose-limiting activity of CAR-T cells against organoids expressing the target of interest. The high level of cultures’ homogeneity ensured by our system allows us to precisely control the effector-to-target ratio, demonstrating the potential of our approach for applications in immunotherapy toxicity and efficacy assessment.

METHODS

GI organoids were pre-labelled with a cell tracker dye and grown on Gri3D® for 3 days. Successively, CAR-T cells and control untransduced T cells (UT) were stained with a fluorescent dye before being seeded onto the organoids at varying densities based on E:T ratios. After 2 days of co-culture, a live/dead (L/D) assay was performed using Calcein-AM (C-AM) and Ethidium Homodimer-I (EthD) to stain the organoids. The fluorescence images were analyzed using Doppl’s automated toxicity assessment pipeline to accurately quantify the degree of cytotoxicity.

RESULTS

Gastrointestinal (GI) organoids were co-cultured with CAR-T cells on Gri3D®. By using fluorescent dyes, we could monitor and track the interactions between the cells, providing important data on their behavior and dynamics. Notably, we observed that the CAR-T cells start to reorganize and attach to the exterior of the organoids immediately after seeding (Fig. 1). The cytotoxic effect of specific CAR-T cells against target-expressing GI organoids was determined through L/D staining and automated quantification (Fig. 2A), confirming that only incubation with target-specific CAR-T and not with untransduced T cells induces cytotoxicity. To validate the specificity of the assay, we conducted parallel tests on both target-positive and target-negative cells, confirming the presence of the specific target in the organoid model (Fig. 2B). Additionally, we analyzed various effector-to-target (E:T) ratios to quantify the dose-response activity of CAR-T cells against target-expressing GI organoids (Fig. 3).

CONCLUSIONS

- Our Gri3D®-based co-culture system is a relevant model for investigating the interplay between immune cells and GI organoids, allowing for precise control over effector-to-target ratios.
- Fluorescent pre-labelling and live imaging techniques enable visualization and quantification of cytotoxic effect of CAR-T cells on GI organoids.
- This novel approach has the potential to contribute to the development of improved immunotherapeutic strategies in the future.

REFERENCES


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