DEVELOPMENT AND CHARACTERIZATION OF A CAR T CELL POTENCY ASSAY WITH 3D CANCER SPHEROID MODELS

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Background The tumor microenvironment poses a significant challenge to immune therapies, like CAR T cells, for solid tumor indications. Development of in vitro 3D models may provide a more accurate assessment of CAR T potency. Here, we developed a real-time, label-free potency assay for evaluation of CAR T cell-mediated cytotoxicity of 3D cancer spheroids.

Methods In this study, the Maestro Z impedance system was used to develop a real-time, label-free potency assay for immune-cell mediated killing of 3D cancer spheroids. Cancer spheroids were produced with SKOV3 cells in ultra-low attachment U-bottom microplates for four days and then transferred to the CytoView-Z plate. The attachment and growth of SKOV3 spheroids was monitored using the resistance measurement of the Maestro Z. After 24 hours, the SKOV3 spheroid and monolayer groups were treated with HER2-specific CAR T cells at matched effector-to-target cell ratios and cytolysis was computed for the following 72 hours of real-time measurement.

Results The spheroid potency assay was used to compare CAR T cell-mediated cytolysis of SKOV3 spheroids and monolayer cultures with HER2-specific CAR T cells. The steady increase in resistance for the untreated group indicated continued spheroid growth after transfer to the CytoView-Z 96-well plate. CAR T effector cells were added at five E:T ratios at 24 hours post-plating of the target cells. The monolayer groups (gray) had higher rates of cytolysis than their respective spheroid groups (orange) for the same E:T ratios (figure 1A). Cytolysis was compared at 72 hours post-addition of the CAR T effector cells (figure 1B) and potency was quantified via a dose response regression. The EC50 E:T ratio of the HER2-specific CAR T cells was 1:3 for the monolayer target cells and 1:1 for the spheroid target cells. These results suggest a decreased potency of HER2-specific CAR T cells against a 3D tumor model in vitro. CAR T potency was also evaluated via the KT50, a measure of the kinetics of immune cell-mediated killing (figure 1C). There was a dose dependence of CAR T killing within the monolayer and spheroid groups, with monolayer groups having shorter KT50s than the matched spheroid groups.

Conclusions These results suggest that 3D cancer spheroid had a higher resistance to CAR T killing than monolayer cultures, which may reflect an improved representation of the tumor microenvironment for an in vitro potency assay.

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(A) Cytolysis over time of 10k SKOV3 spheroids and monolayers treated with 4-1BB (HER2 targeting) CAR T cells at effector-target ratios of 1:10, 1:5, 1:2, 1:1, and 5:1. (B) Cytolysis between spheroids and monolayer in respect to their effector-target ratios. EC50 measured the 4-1BB CAR T ratio required to achieve 50% cytolysis. (C) KT50 between spheroids and monolayer groups, displaying both dose dependence for both groups and differences in duration to achieve 50% cytolysis between groups. *indicated 1:2 spheroid group did not reach a KT50.