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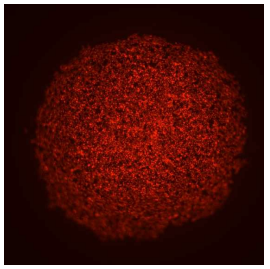
Time-lapse killing assay (spheroid - IncuCyte) V.2



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Philippa R Kennedy[1], Peter Hinderlie[1]

[1]University of Minnesota

Miller Group - imaging an…

Philippa R Kennedy
University of Minnesota

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Protocol status: Working
We use this protocol and it's working

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Abstract

Many tumors exist *in vivo* as three-dimensional masses. In order to better model the dynamics of three-dimensional tumor growth and immune cell invasion, cancer cell lines are grown in low-adhesion plates that force them to interact with each other, forming a spheroid. After spheroids form in culture, immune effectors and drugs are added to the wells and their effect on the tumor mass is measured by transmitted light or fluorescence microscopy in a live-cell image system (IncuCyte, Sartorius Inc).

Guidelines

We do not use an indicator of cell death (e.g. Caspase 3/7 Green) as standard in this assay, because tumor fluorescence is generally sufficient for monitoring tumor growth. Cells that divide rapidly will lose fluorescence if labelled with cell trace, which will make analysis complicated. It is therefore preferable to use a fluorescent gene construct in the tumor cell as opposed to cell trace labeling.

The following cell lines will stable form spheroids using the main protocol in the indicated time frame:

H2373-GFP spheroids - takes 3 days to form (round but loose).
H2461-GFP spheroids - takes 1 day to form (very small and neat).
H2596-GFP spheroids - takes 2 days to form (small but irregular).
HAY-GFP spheroids - takes 3 day to form (messy but analyzable with a high fluorescence threshold).
HT29-NLR spheroids - takes 4 day to form (messy but analyzable with a high fluorescence threshold).
Cal27-NLR spheroids - takes 3 days to form (very round and neat).
Cal33-NLR spheroids - takes 3 days to form (very round and neat).

A549 require a distinct spin protocol in order to form:
First, spin the plate up to 50 *g* (500 RPM), acceleration 5, brake 0 in one orientation and allow to come back to a stop once the centrifuge reaches speed. Then spin up to 100 *g* (700 RPM), acceleration 5, brake 0 in the opposite orientation and allow to come back to a stop once the centrifuge reaches speed.

Enriched NK cells are usually added at 5:1 effector:target ratio. PBMC's should be run at 20:1 effector:target ratio if NK cells are the primary effectors within the mixture.

Materials

ULA plate, Cat. No. 7007, Corning
Microscope within incubator e.g. IncuCyte, Sartorius Inc
Fluorescent tumor cell
Effector cells
Drugs

1 *Optional:* If target cells that form a spheroid are to be themselves monitored by fluorescence, perform this labeling/transfection in advance (see ***Time-lapse killing assay - monolayer - Incucyte***)

2 Resuspend target cells in their preferred media. Seed $2x10^4$ target cells in 100 µL/well into a 96 well U-bottom low adhesion plate (ULA plate, Cat. No. 7007, Corning).

3 Set the plate to spin in a centrifuge at 200 *g* (1000 RPM), acceleration 6, brake 0. Once the plate has reached this maximum speed, stop the spin and allow it to come to a gentle stop (~10 min). Then remove the plate, and repeat the procedure with the plate rotated 180° in the horizontal plane to ensure no skewing of the pellet.

4 Capture an image of the plate using the live-cell image system (Incucyte S3 or Zoom, (Incucyte S3) to ensure the cells are in the center of the well. If cells are at the edges, contain large gaps in the pellet or are too tightly condensed, resuspend them with a multichannel pipette (breaking up the pellet) and repeat the seeding, altering the centrifugal forces until a central single-layered pellet is achieved.

5 After allowing spheroids to form for 1-5 days, add effector cells and treatments on top of the spheroids up to a total well volume of 200 µL. **It is important to do this very slowly and precisely as the spheroids are not attached to the plate.**

6 Replace in the plate in the live-cell image system and take images of the wells at regular intervals for 5 days.

7 Image analysis is performed in the IncuCyte software. The size of each spheroid, as detected by edge analysis of the transmitted light image or fluorescence detection of the spheroid mass, is measured for each time point and normalized to the size of the spheroid at the time effectors and drugs were added (t = 0 h).