Noble Agar Assay for Self-Renewal

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Method Article

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Abstract

The noble agar assay is based on the principle that the cancer cells are anchorage-independent and do not show contact inhibition. This type of soft agar assay can give insight into two important properties of a subset of cancer cells. Firstly, the tumorigenic efficiency of cancer cells can be determined based on number of colonies that emerge. This is based on the ability of cells to grow unattached and to remain suspended in agar. Secondly, the self-renewal properties of cancer cells can be assessed based on the types of progeny produced in noble agar.

Reagents

Noble agar \(Sigma A5421-250G) 60mm Petri dishes Sterile deionized water 2X DMEM powder \(phenol-red-free DMEM for fluorescence applications) Fetal bovine serum Cancer cells \(primary or cell line) of choice trypsin-EDTA PBS

Equipment

55°C water bath Microscope \(fluorescent microscope if necessary) Autoclave

Procedure

1. Prepare stock of 1.8% noble agar in sterile deionized water. 1a. Add 1.8 g noble agar to 100ml deionized water. 1b. Autoclave at 121°C for 15 min. 1c. Keep this solution in a 55°C water bath until ready to use. 2. Prepare 2X DMEM. 2a. Add 1X DMEM powder \(originally intended to make 1L solution) into 500ml deionized water. 2b. Sterile filter this solution and place at 37°C \(to prevent premature solidification of noble agar when noble agar is added). 3. Prepare cancer cells \(to be mixed into top layer of noble agar in Step 5). 3a. Wash cancer cell cultures twice in 1X PBS. 3b. Detach cells \(for cultured cell lines) or dissociate cells \(for primary tumors) using trypsin-EDTA, harvest via centrifugation, and count cells. 3c. Dilute appropriately to add log10-fold dilutions of cells to 1.33ml sterile PBS in microcentrifuge tubes. For example, add 1 cell to a tube with 1.33ml PBS, add 10 cells to a tube with 1.33ml PBS, add 100 cells to a tube with 1.33ml PBS, etc. 3d. Set this cell solution aside until ready to seed \(see step 5). 4. Plate the bottom agar \(final concentration 0.6% noble agar, 1X DMEM, total volume 4 ml) into 60mm Petri dishes as follows: 4a. In a sterile 10ml tube, mix 2ml of 2X DMEM with 1.33ml of 1.8% noble agar and 0.67ml sterile deionized water. 4b. Immediately pour this 4ml solution onto 60mm Petri plates. Perform in triplicates. 4c. Allow plates to solidify at 37°C for 10 min. 5. Plate the top agar \(final concentration 0.3% noble agar, 1X DMEM, total volume 4 ml) as follows: 5a. In a sterile 10ml tube, Mix 2ml of 2X DMEM with 0.67ml of 1.8% noble agar and 1.33ml cell solution from Step 3c). 5b. Immediately pour this 4ml solution onto the bottom layer of 60mm Petri plates from Step 4. Perform in triplicates. 6. Place plates at 37°C overnight to allow for colony formation. 7. Using a microscope, count the number of colonies on each 60mm plate. 7a. Determine the tumorigenic efficiency based on the number of cells

plated: Efficiency = \(# of colonies)/\(# of cells plated) x 100% 7b. Determine the types of progeny produced \(qualitative assessment by visualization).

Timing

This assay will take approximately 3 hours.

Troubleshooting

Critical Steps Step 2b: Be sure to keep DMEM at 37°C prior to use. If noble agar solution is added to cold \(or even room temperature) DMEM, solidification will begin too soon and you will not be able to proceed with the assay. Step 4b: Be sure to immediately pour this solution onto the plates, otherwise the solution will solidify in the 10cc tube. Step 5b: Be sure to immediately pour this solution onto the plates, otherwise the solution will solidify in the 10cc tube.

Anticipated Results

The most primitive cancer cells are expected to have the highest tumorigenic efficiency. The most primitive cancer cells are expected to generate both primitive cells and differentiated cells. Cancer cells that lack stem-like properties may initially form colonies, but these colonies are expected to regress due to lack of self-renewal ability. Normal epithelial cells \(such as non-tumorigenic MCF12A breast cells) are not expected to form any colonies.

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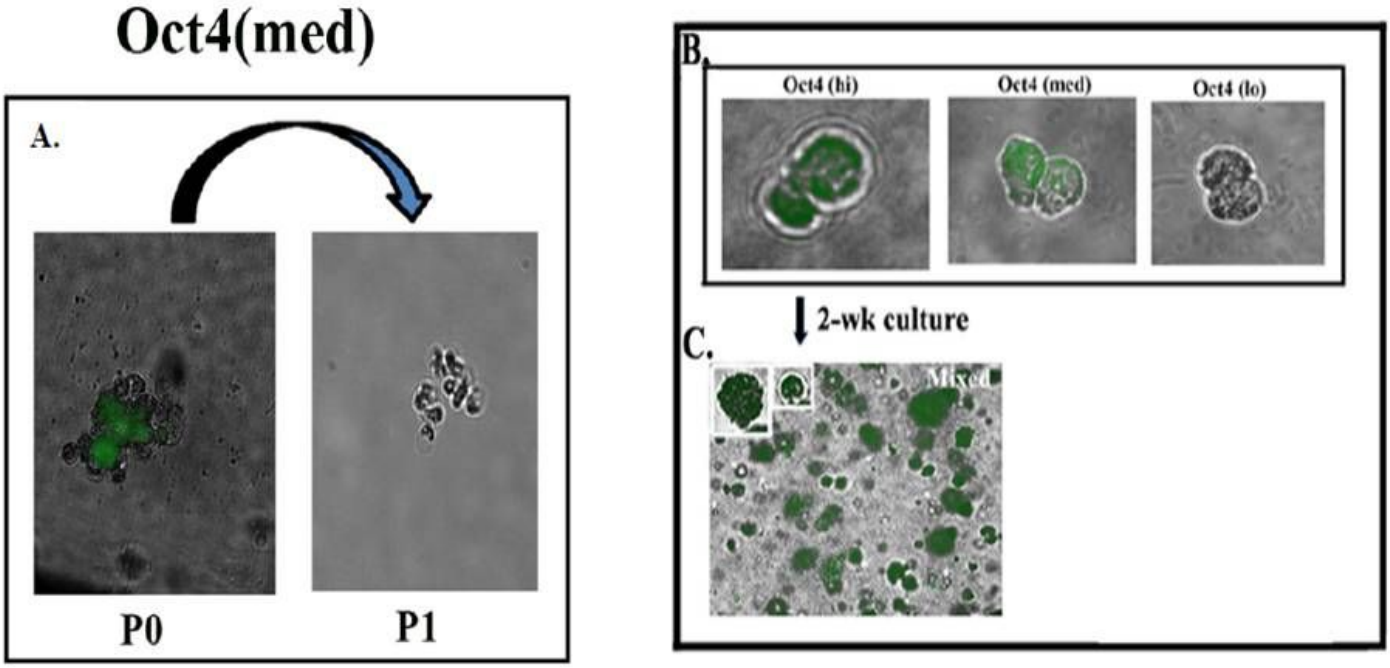
Figures



Relative maturity of BCC subsets. A) The figure shows a small tumorsphere from Oct4^{med} BCCs that failed to be serially passaged. **B)** Shown are the first doubling times for different BCC subsets. **C)** The parental and daughter from an original Oct4^{hi} cell were expanded in liquid culture. At wk 2, the cells were subcultured in noble agar. Inset shows the parental and second generation daughter cells.

Figure 1

Noble agar assay