G236p53DD/E1A Culture

Kazuhide Watanabe

6-21-2011

Summary

G236p53DD/E1A was isolated from a mouse mammary tumor with MMTV-Wnt1/Py2$^{flox/-}$ genotype (mouse No. G236, B6/FVB/N mixed strain, established by KW). The isolated cells were cultured in 3D to form tumorsphere and then plated onto a plastic 2D dish, followed by the retroviral transduction of Dominant negative p53 (p53) and adenoviral E1A (pBABE-hygro p53 DD and pWZL hygro 12S E1A). Therefore, this line is hygromycin resistant. A bulk population of survived cells was established as a cell line. Pygo2 gene can be excised by exogenous Cre and Pygo2 protein was completely undetectable three days after Cre-adenovirus infection.
A stable Wnt reporter line is also available. (lentiviral construct of TOP-flash 7TFP, puromycin resistant) (Fuerer and Nusse, 2010).

Culture Medium preparation

1. In 1 liter beaker with a stir bar, add

800 ml milliQ water	
DMEM/F12 (1:1) powder (12500-062, Invitrogen)	1 bag
HEPES powder (BP310-500, Fisher)	5.95 g (25 mM final)
NaHCO3 powder (S233-500, Fisher)	2.438 g
Pen-Strep aliquot (100x) (15140-122, Invitrogen)	10 mL

2. Adjust pH to 7.1-7.2 with 0.5M NaOH while stirring

3. Bring volume to 900 ml

4. Add the following:

Insulin (5 mg/ml in PBS) (I6634-250MG, Sigma)	1 ml (5 µg/mL final)
EGF (100 µg/ml) (01-107, Millipore)	50 µL (5 ng/mL final)
Hydrocortisone (4mg/ml in ethanol) (386698, Calbiochem)	250 µL (1 µg/ml final)
Heat-inactivated FBS (varies)	100 ml (10% final)

5. Mix well again by stirring

6. Filtered through 500 ml cup filter (0.22 µm, SCGP-T05-RE, Millipore) to TC bottles (200 ml/bottle)

7. Working media at 4 C; freeze unused media – can be stored in -20 ºC for months before use

Routine culture (for 100-mm dish)

Feed with fresh medium every 2-3 days. Usually split the cells every 3-5 days at 1:10 dilution.
Note: these cells start dying rapidly after reaching confluency.

1. wash twice with Ca^{2+}-free PBS (5-10 mL).

2, add 2 mL of 0.25% Trypsin/EDTA and incubate for 3-5 min at 37ºC.

3, Neutralize Trypsin by adding 5-mL of culture medium, then mix well, and centrifuge at 1000 rpm for 5 min.

4, aspirate supernatant and split the cells with 8-10 mL of fresh medium/dish.

Frozen stocks

Resuspend 1-2 million cells in 1ml culture media with 10% DMSO, store in sealed Styrofoam at -80ºC for at least overnight, then transfer to plastic box in liquid nitrogen tank.

Adenoviral infection (12-well plate)

Day-1. Plate $1x10^5$ cells into a 12-well plate.

Day0. 1. Prepare $5x10^6$ TU (transduction unit, based on 293T cells) adenoviral solution by adding growth medium up to 200 µL in total.

2. Aspirate medium from the cells and add adenoviral solution. Rock the plate to ensure that the entire surface area is covered.
3. Incubate the cells for 60 min in CO_2 incubator, rocking the plate every 15 min.
4. after 60 min, add 1 mL of growth medium.

Day1. replace medium or split cells.

Day2~ Confirm Pygo2 expression and perform experiments.

References

Fuerer, C., and Nusse, R. (2010). Lentiviral vectors to probe and manipulate the Wnt signaling pathway. PloS one *5*, e9370.