Data Sheet

Mouse HPV16 E6/E7/hRas (mEER) Cell Line

SCC626

Pack Size: ≥ 1x10^6 viable cells/vial

Store in liquid nitrogen.

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for human or animal consumption.

Background

The head and neck cancers represent the sixth most common class of cancer.[1,2] Among them, incidents of the oropharyngeal squamous cell carcinoma (OPSCC) are on the rise, and a majority of them are caused by the human papilloma virus (HPV).[3] While successful management methods exist, the local recurrence and the nodal metastasis do result in mortality in a minority of the cases. Historically, insights into the HPV-induced transformation have been obtained in the context of the cervical cancer and the anogenital warts models, and, while much of it is relevant to OPSCC, it will be beneficial to have the systems in which the effects of HPV infection can be studied in the context specific to OPSCC.

The Mouse E6/E7/hRas (mEER) cell line, was established by the retroviral transduction of the HPV E6 gene, the HPV E7 gene, and the human hRas G12V gene into the primary mouse oropharyngeal epithelial cells.[4] It had been shown that HPV E6 and E7 promote the clearance of the G1 checkpoint by destabilizing the TP53 protein and the Rb protein. It was furthermore found that co-expression of the hRas protein synergized with these HPV genes to confer a markedly more invasive phenotype. The resulting cell line was capable of seemingly indefinite cultivation and anchorage-independent growth i*n vitro*. The mEER Cell Line expressed many of the hallmark genes of OPSCC, including cytokeratin, E-cadherin, BRCA2, p16, and EGFR, and formed poorly-differentiated, aggressively invasive tumors following orthotopic injection in immunocompetent mice.[4] The derivatives of these cells (also available as SCC627 and SCC625) were found to spread through the lymphatic space to the lungs, which is anatomically relevant to human oral cancers and makes the mEER Cell Line a suitable platform for mechanistic studies and testing therapeutic intervention strategies.[5, 6]

Source

The mEER Cell Line was established by the retroviral transduction of the HPV16 E6 gene, HPV16 E7 gene, and the human hRas gene into the primary tonsilar epithelial cells isolated from a male C57BL/6 mouse.[4]

Short Tandem Repeat

M18-3	17	M1-2	18	M8-1	16	M11-2	16, 17	MX-1	28
M4-2	20.3	M7-1	26.2	M2-1	16	M17-2	15	M13-1	17
M6-7	15	M1-1	15, 16	M15-3	22.3	M12-1	17		
M19-2	13	M3-2	15	M6-4	18	M5-5	17		

Quality Control Testing

- The mEER cells are verified to be of mouse origin and negative for human, rat, Chinese hamster, Golden Syrian hamster, and non-human primate interspecies contamination, as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells tested negative for infectious diseases against a Mouse Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

The mEER cells should be stored in liquid nitrogen until use. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting the cell marker expression and functionality.

Representative Data

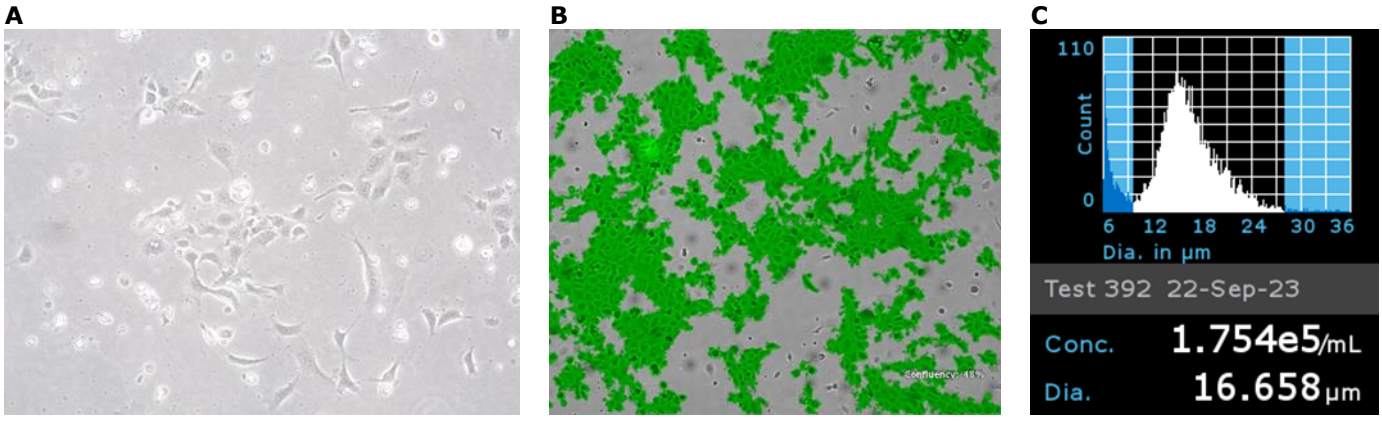

Figure 1. (**A**) Bright-field images of mEER cells a day after thaw in a T175 flask. (**B**) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (MDCI10000). (**C**) Cell counting was performed using Scepter™ 3.0 Handheld Automated Cell Counter using 60 µm sensor tips (PHCC360KIT).

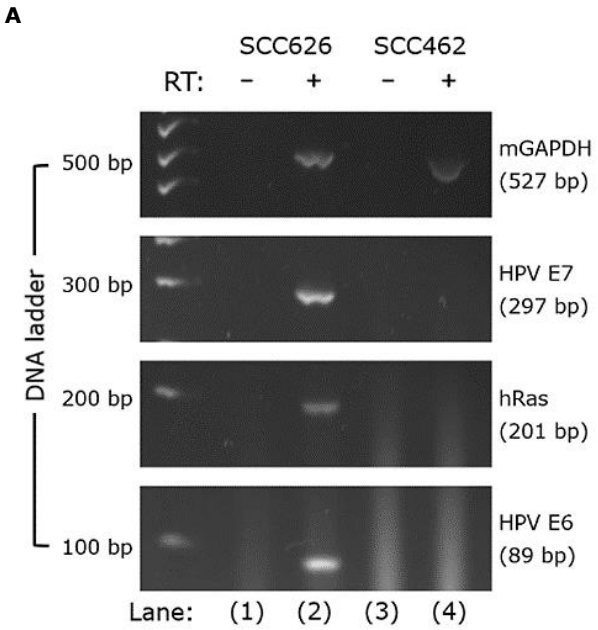

Figure 2. Transgene expression in the mEER Cell Line, the mouse EER cell line. (**A**) End-point RT-PCR with primers specific for HPV16 E6 (expected product size 89 bp), HPV16 E7 (expected 297 bp), and human hRas (expected 201 bp) successfully detected these transcripts, when the total RNA prepared from the mEER Cell Line was pre-incubated with reverse transcriptase (Lane 2) but not without the RT enzyme (Lane 1). These transgenes were not detected in an untransfected (and unrelated) mouse cell line, with or without RT (SCC462, Ret-melanoma Sorted cell line, Lanes 3 and 4). Mouse GAPDH (expected 527 bp) was used as control. The TaqMan RNA-to-CT 1-Step Kit (Applied Biosystems 4392938) was used for the reactions.

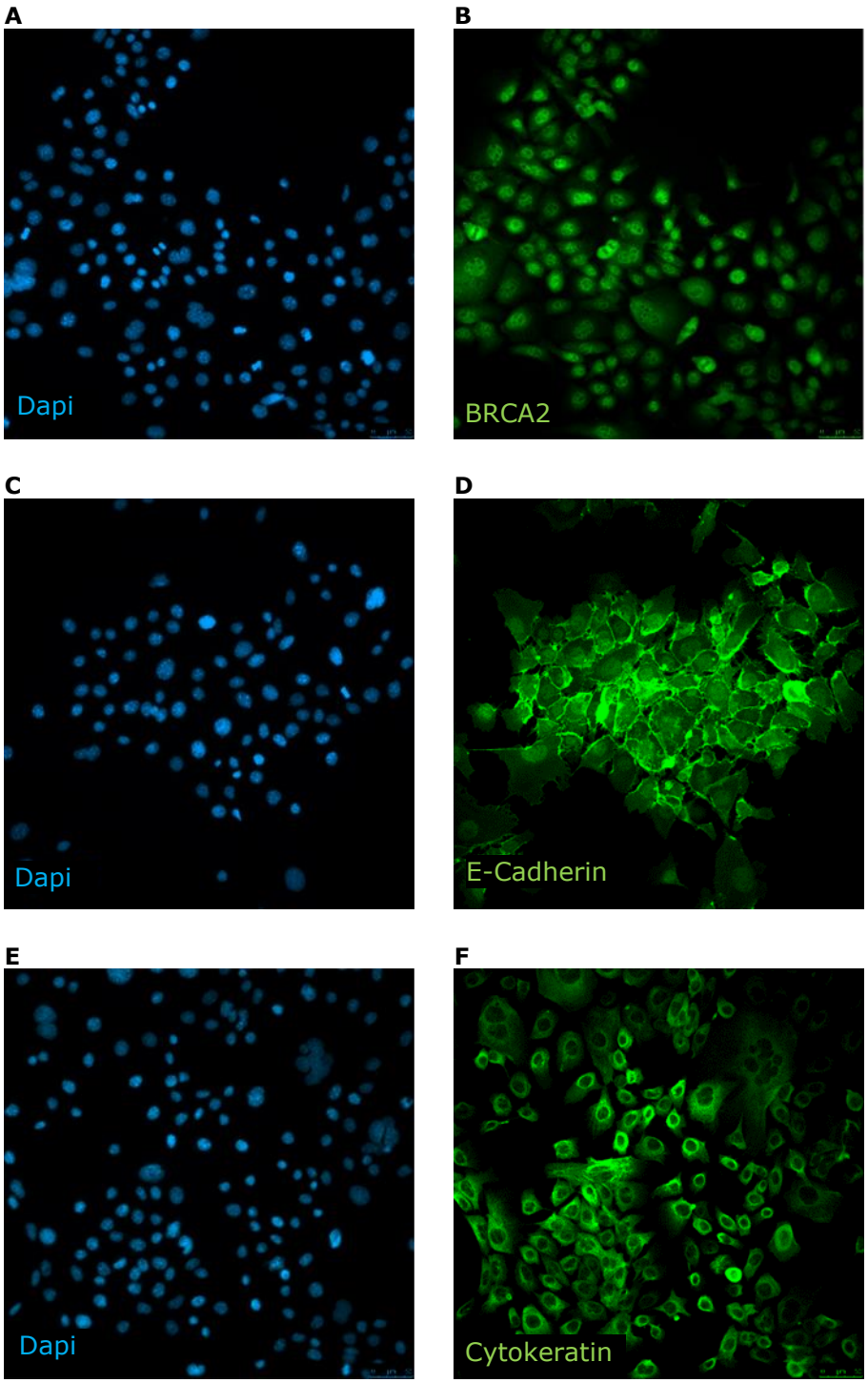

Figure 3. The mEER cells express hallmark genes of oropharyngeal squamous cell carcinoma. (**A, C, E**) mEER cells labeled with DAPI (MBD0015), (**B**) anti-BRCA2 antibodies (HPA026815), (**D**) anti-E-Cadherin antibodies (SAB5700789), and (**F**) anti-cytokeratin antibodies (C2931).

Protocols

The mEER cells proliferate rapidly. We recommend thawing in a T175 flask and use of trypsin to detach the cells.

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue culture ware surfaces without any additional coating. mEER cells are thawed and expanded in mEER Expansion Medium comprising of DMEM/F12 (DF-042-B) containing 10% FBS (ES-009-B), 0.5 µg/mL hydrocortisone (H0888), 5 µg/mL human apo-Transferrin (T4382), 5 µg/mL human insulin (I9278), 5 ng/mL human Epidermal Growth Factor (SRP3027), 1.36 ng/mL triiodothyronine (T6397), with optional Penicillin/Streptomycin (P4333).
2. Remove the vial of frozen cells from liquid nitrogen and incubate in a 37 °C water bath. Closely monitor until the cells are completely thawed. Maximum cell viability is dependent on the rapid and complete thawing of frozen cells.

   Important: Do not vortex the cells.
3. As soon as the cells are completely thawed, disinfect the outside of the vial with 70% ethanol. Proceed immediately to the next step.
4. In a laminar flow hood, use a 1 or 2 mL pipette to transfer the cells to a sterile 15 mL conical tube. Be careful not to introduce any bubbles during the transfer process.
5. Using a 10 mL pipette, slowly add dropwise 4 mL of mEER Expansion Medium (medium composition in Step 1) to the 15 mL conical tube.

   Important: Do not add the entire volume of media all at once to the cells. This may result in decreased cell viability due to osmotic shock.
6. Gently mix the cell suspension by slowly pipetting up and down twice. Be careful not to introduce any bubbles.

   Important: Do not vortex the cells.
7. Centrifuge the tube at 300 x *g* for 5 minutes to pellet the cells.
8. Decant as much of the supernatant as possible. Steps 5-8 are necessary to remove residual cryopreservative (DMSO).
9. Resuspend the cells in 40 mL of mEER Expansion Medium.
10. Transfer the cell mixture to a T175 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. The mEER cells can be passaged at ~80-85% confluency.
2. Carefully remove the medium from the T175 tissue culture flask containing the 80-85% confluent layer of cells.
3. Rinse the flask with 30-40 mL 1X sterile PBS (TMS-012-A). Aspirate after the rinse. Repeat this wash step one more time.
4. Apply 10 mL of 0.25% Trypsin-EDTA pre warmed to 37 °C (SM-2003-C) and incubate in a 37 °C incubator for 5-7 minutes.
5. Inspect the flask and ensure the complete detachment of cells by gently tapping the side of the flask with the palm of your hand.
6. Add 10-15 mL of mEER Expansion Medium to the flask.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 50 mL conical tube.
8. Centrifuge the tube at 300 x *g* for 5 minutes to pellet the cells.
9. Discard the supernatant, then loosen the cell pellet by tapping the tip of the tube with a finger.
10. Apply 2-5 mL of mEER expansion medium to the conical tube and resuspend the cells thoroughly. Large cell clumps may be broken up by gentle trituration.

    Important: Do not vortex the cells.
11. Count the number of cells using a hemocytometer or a Scepter 3.0 Handheld Automated Cell Counter.
12. Plate the cells to the desired density. Typical split ratio is 1:10.

Cryopreservation of the Cells

The mEER cells may be frozen in 90% fetal bovine serum (FBS) with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Kademani D. 2007. Oral cancer. Mayo Clin Proc. 82(7): 878-87.
2. Rogers SN, Brown JS, Woolgar JA, Lowe D, Magennis P, Shaw RJ, Sutton D, Errington D, Vaughan D. 2009. Survival following primary surgery for oral cancer. Oral Oncol. 45(3):201-11.
3. Pytynia KB, Dahlstrom KR, Sturgis EM. 2014. Epidemiology of HPV-associated oropharyngeal cancer. Oral Oncol. 50(5): 380-6.
4. Hoover AC, Spanos WC, Harris GF, Anderson ME, Klingelhutz AJ, Lee JH. 2007. The role of human papillomavirus 16 E6 in anchorage-independent and invasive growth of mouse tonsil epithelium. Arch Otolaryngol Head Neck Surg. 133(5): 495-502.
5. Williams R, Lee DW, Elzey BD, Anderson ME, Hostager BS, Lee JH. 2009. Preclinical models of HPV+ and HPV- HNSCC in mice: an immune clearance of HPV+ HNSCC. Head Neck. 31(7): 911-8.
6. Vermeer DW, Coppock JD, Zeng E, Lee KM, Spanos WC, Onken MD, Uppaluri R, Lee JH, Vermeer PD. 2016. Metastatic model of HPV+ oropharyngeal squamous cell carcinoma demonstrates heterogeneity in tumor metastasis. Oncotarget. 7(17): 24194-207.

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