Product description

Keratinocyte cell line representing the ultimate metastatic stage in a SCC cancerous transformation. MET4 is derived from a from metastatic SCC within left axillary lymph nodes that were biopsied following the third excision. The cell lines originated as a primary tumour on the left hand. Histology of the original tumour showed an acantholytic squamous cell carcinoma. MET4 has been used to understand aggressive metastatic SCCs and is an important cell line for this specific stage in SCC cancer progression. MET4 is the fourth cell line in a unique series of epidermal cell lines representing different stages of malignant transformation. These cell lines were derived from a single, adult, immunosuppressed individual. Four keratinocyte lines (PM1-4) established from forehead skin are here compared with 4 squamous cell carcinoma (SCC) lines MET2 derived respectively from a primary cutaneous tumour, two local recurrences and a distant metastasis of invasive SCC. Despite altered growth properties, the PM lines retained many features of normal keratinocytes including keratin phenotype, differentiation capacity and non-tumorigenicity in athymic mice. In contrast, from early passage, the MET lines displayed markedly reduced growth requirements, abnormal differentiation, aberrant K18 expression and tumorigenicity in athymic mice. The abnormal keratin profile of individual MET lines closely reflected the keratin phenotype of the tumour of origin. Although unusual HPV types were identified in the original tissue, there was no evidence of persistent virus within any cell line and it appears that HPV is not critical for maintenance of the immortal phenotype. The PM lines were distinctly different from invasive SCC lines and are likely to be useful in studies focusing on mutations that are important in early neoplastic progression. The SCC series represent primary, recurrent and metastatic carcinoma. Availability of such a series from the same individual will facilitate genetic analysis of the malignant process.

Name: MET4 SCC cell line

Cancer: Skin Cancer

Cancers detailed: Squamous Cell Carcinoma

Organism: Human

Gender: Male

Tissue: metastatic SCC within left axillary lymph nodes biopsied following the third excision.

Donor: 46 year old immunosuppressed patient

Growth properties: Adherent

Model: Tumour cell line

Model description:

CRISPR Edited: No

Production details: MET4 was derived from metastatic SCC within left axillary lymph nodes biopsied following the third excision of a tumour originating in the left hand, as described in Hassan et al., 2019. PMID: 31336867

Biosafety level: 1

Cellosaurus id: CVCL_LN12

Contributor(s)

Inventor: Irene Leigh
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Properties

Product format: Frozen

Unpacking and storage:

1. Check all containers for leakage or breakage.

2. Remove the frozen cells from the dry ice packaging and immediately place the cells at a temperature below -130°C, preferably in liquid nitrogen vapour, until ready for use.

Recommended medium: Keratinocyte medium (3:1 V/V mixture of DMEM and Ham's F12) supplemented with 10% (K)FBS + and a cocktail of mitogens: hydrocortisone (0.5 µg/ml), cholera toxin (10^{-10} M), EGF (10 ng/ml) and insulin (5 µg/ml) as well as with 100 IU/ml penicillin, and 100 µg/ml streptomycin. Also add Tri-iodothyronine (500 ul) solution (2 nM final concentration). Complete keratinocyte growth medium is then stored at 4°C and is ideally used within 4 weeks.

Cell Density: Cells should be seeded at a density of 2–3 x 10^5 per T75 flask containing a recently prepared feeder layer. The seeding density needs to be optimised for different strains of cSCC cell lines with different cell division rates.

Culture conditions: 37.0°C ± 1.0°C incubator with 5.0% ± 1.0% CO_2

Handling instructions

Please ensure that vials are frozen when received, and store at **<-130 °C long term**. When removing frozen cells from storage, it is important to minimize exposure to room temperature (15 - 25°C). If not proceeding directly to thawing, place the cells on dry ice or in a liquid nitrogen container.

Preparing the Feeder Layer (for more details see separate 3T3-J2 culture protocol)

1. To irreversibly inhibit proliferation of the 3T3-J2 feeder cells, add mitomycin C to a 70–90% confluent (T75 or T175) flask of 3T3-J2 fibroblasts (final concentration, 4 µg/ml, usually 1:100 from 100x stock) and incubate for 3 hr at 37°C, 7.5–10% CO_2.
2. Remove the medium, rinse the cells once with 0.02% (w/v) EDTA (Versene), then harvest cells in 0.05% (w/v) Trypsin plus 0.02% (w/v) EDTA (Trypsin-EDTA; made by diluting 0.25% (w/v) Trypsin plus 0.02% (w/v) EDTA 1:5 in Versene solution, usually 1 ml of 0.25% (w/v) Trypsin plus 0.02% (w/v) EDTA and 4 ml Versene solution for a T75 flask; double the volumes for a T175 flask)). Centrifuge 3 min (1,200 rpm, RT; depending on the type of centrifuge, this will be~ 1000 x g) to pellet cells. Aspirate supernatant, resuspend cells in fresh fibroblast growth medium, and count cell numbers. The optimal density of the feeder layer is 3 x 10^6 cells per T75 flask and 6 x 10^6 cells per T175 flask.
3. The feeders can be used immediately (i.e., plated at the same time as the keratinocytes) or prepared a day before they are required; if prepared in advance they should be maintained in fibroblast growth medium (DMEM + 10% bovine serum) at 37°C, 5% CO_2. The feeder layer should not be prepared more than 2 days before us because the feeder cells will start to degenerate.

Reviving frozen cells:

1. **Do not thaw at room temperature.** To thaw, swirl the vial quickly in a 37 °C water bath with O-ring and cap above the water to avoid contamination. Remove from the water bath with a small ice pellet remaining

(this should not take more than 2 minutes) and wipe the exterior with 70% ethanol or isopropanol before transferring to a biosafety cabinet. Further steps should be conducted under aseptic conditions.

2. We strongly recommend that the volume of cell suspension is measured at this point, and a 20 uL aliquot be removed for a **viable cell count** using trypan blue or similar dye. This ensures that provided cells are viable, and the cell count can be used to determine volume of growth medium to be added to the cell suspension.
   Note: a healthy culture of keratinocytes (normal and neoplastic) will contain a majority of small, round cells in the suspension when counting; larger cells are likely cells that have undergone terminal differentiation, or residual fibroblasts.
3. Centrifuge the cell suspension at **1000x g for 3 minutes** at room temperature.
4. Carefully remove the supernatant with a pipette, leaving a small amount of medium to ensure the cell pellet is not disturbed. Resuspend the cell pellet by gently flicking the tube.
5. Cells should be seeded at a density of 2–3 x 10^5 per T75 flask containing a recently prepared feeder layer. The seeding density needs to be optimised for different strains of cSCC cell lines with different cell division rates.
6. Change the medium every 2–3 days. Passage cells once the colonies are just about to begin to touch each other (usually after a week).

Subculture steps (T75 flask):

1. Passage cSCC cells prior to confluence (colonies should not touch each other). This is especially important for normal keratinocytes, as increasing numbers of cells will undergo terminal differentiation post-confluence.
2. Remove the medium and rinse the cultures once with 5 ml of 0.02% (w/v) EDTA (Versene).
3. Add fresh 5 ml of Versene solution and incubate for 5–10 minutes at 37°C, 5% CO_2 to selectively detach the feeder cells. Detach feeder cells by vigorously rapping the side of the flask with the palm of the hand. Gently flush with the Versene solution over the feeder layer using a pipette, and afterwards aspirate the solution. Wash once more with 5 ml of Versene solution. If necessary, repeat this procedure 2–3 times more (1 min incubations at 37°C), until the feeder cells have been completely removed and the cancer cell colonies become clearly visible against the plastic surface.
   Note: Detaching the feeder cells should be complete after max. 20 mins. If feeder cells tend to stick tightly to the keratinocyte colonies, this likely indicates that the 3T3-J2 fibroblasts have started to undergo transformation.
   Note: To speed up the detachment of the feeder cells in case they are very sticky, 5 ml of Versene solution supplemented with 100 µl of 0.25% (w/v) Trypsin plus 0.02% (w/v) EDTA can be used.
   Note: Compared to normal human keratinocytes, neoplastic keratinocytes often display reduced adhesion to the cell culture surface. Thus, they should be more carefully monitored during removal of the feeder layer, to not lose the cells.
4. To detach the cSCC cells, add 4 ml Versene solution and 1 ml 0.25% (w/v) Trypsin plus 0.02 (w/v) EDTA to the flask and incubate at 37°C for about 5–10 min. Detach cSCC cells by vigorously rapping the flask.
5. Add 5ml of complete medium to inactivate the trypsin. Thoroughly rinse the adhesive area of the flask and re-suspend the cells gently but well by pipetting to disrupt cell aggregates. Filter the cell suspension once through a 40 µm cell strainer attached to a 50 ml centrifuge tube (to remove cell aggregates).
6. Centrifuge at 1000x g for 3 minutes at room temperature to recover cells and re-seed at the recommended density.

Cryopreservation:

Solution:
10% (v/v) DMSO/ 90% (v/v) (K)FBS; sterile filtered.

Steps

1. For freezing down cell stocks, harvest the cSCC cells as described above but re-suspend at 1 x 10^6 cells/ml in cryo-preservation solution (10% (v/v) DMSO plus 90% (v/v) (K)FBS). If the cryo-preservation solution is made fresh, take care to add the DMSO to cold serum, as the resulting

exotherm reaction will heat up the solution.
2. Place 1 ml of cell suspension in each 1.8-ml cryotube.
3. Place the tubes in a Nalgene freezing container ('MrFrosty'; ensure that the container has been freshly filled with correct amount of 100% (v/v) isopropanol) for 24 hours at -80°C and then immediately transfer to liquid nitrogen for long term storage.

References

- Inman et al. 2018. Nat Commun. 9(1):3667. PMID: 30202019.
- Abikhair Burgo et al. 2018. JCI Insight. 3(17):. PMID: 30185657.
- Mekhdjian et al. 2017. Mol Biol Cell. 28(11):1467-1488. PMID: 28381423.
- Proby et al. 2000. Exp Dermatol. 9(2):104-17. PMID: 10772384.

Material Citation

If use of this material results in a scientific publication, please cite the material in the following manner: MET4 SCC cell line, was invented by Irene Leigh (CancerTools.org #153539).