Data Sheet

PyMT-1099 Murine Breast Cancer EMT Cell Line

SCC422

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

PyMT-1099 is a stable murine breast cancer cell line derived from a mammary tumor (mammary gland 2/3) of MMTV-PyMT (mouse mammary tumor virus-polyomavirus middle tumor-antigen) transgenic female mouse of FVB/N background.[2] PyMT is an oncogene which transforms cells in culture and leads to formation of mammary tumor in MMTV-PyMT mouse by interacting with non-receptor tyrosine kinase, c-Src.[1]

These cells have a well differentiated, cobblestone-like morphology similar to epithelial cells and have the PyMT transgene in their genome. Orthotopic implantation of these cells in immunocompromised NOD/SCID, common gamma receptor −/− (NSG) mice resulted in formation of palpable tumor after 14 weeks. 50% of the mice had macroscopic metastatic tumors in their lungs exhibiting invasive characteristics.[2]

Breast cancer is currently the most common cancer globally. In the United States, approximately 287,850 new cases of invasive breast cancer and 43,250 breast cancer-associated deaths were estimated in 2022[3]. Epithelial-mesenchymal transition (EMT) is a process involved in the initiation of the invasion-metastasis cascade of cancer cells. PyMT-1099 undergo epithelial-mesenchymal transition (EMT) in response to TGFβ addition *in vitro* and undergo a complete mesenchymal to epithelial transition (MET) upon TGFβ withdrawal. TGFβ treatment (2 ng/mL) of PyMT-1099 cells resulted in complete reduction of epithelial markers such as E-cadherin and Zona Occludens (ZO-1), while the mesenchymal markers Fibronectin (FN1) and N-Cadherin were significantly and moderately upregulated respectively. as evidenced by immunofluorescence.[2]

Although stimulation with TGFβ was reported to induce the canonical pathway as evidenced by nuclear translocation of the Smad2/3 complex and phosphorylation of Smad3 [4], these cells are resistant to the TGFβ induced cell cycle arrest and utilize non-canonical TGFβ signaling to induce EMT. Long term treatment with TGFβ (2 ng/mL) for 20 days results in formation of mesenchymal subline of cells (PyMT LT) with elongated morphology. PyMT LT cells exhibit higher migratory potential compared to untreated PyMT-1099 cells or NMuMG (immortalized normal murine mammary gland) cells in a modified Boyden chamber transwell migration assay. Also, in contrast to their epithelial counterpart, PyMT-1099 cells, 100% of mice (immunocompromised-NOD) implanted orthotopically with PyMT-1099 LT cells had macroscopic lung metastatic lesions.[2]

Hence, PyMT-1099 cells will be an excellent reliable model that can replicate dynamic “plastic” changes associated with all stages of EMT and MET in cancer cells both *in vitro* and *in vivo*.

Source

PyMT-1099 cells were derived from mammary tumors of MMTV-PyMT transgenic female FVB/N mice.[1]

Short Tandem Repeat

M18-3: 18	M1-2: 16,17	M8-1: 16	M11-2: 14,15	MX-1: 27,28
M4-2: 20.3	M7-1: 25.2	M2-1: 15,16	M17-2: 16,17	M13-1: 16.2,17
M6-7: 15	M1-1: 15,16	M15-3: 21.3,22.3	M12-1: 16	
M19-2: 12	M3-2: 14	M6-4: 17,18	M5-5: 15,16	

Quality Control Testing

- PyMT-1099 murine breast cancer 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

PyMT-1099 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

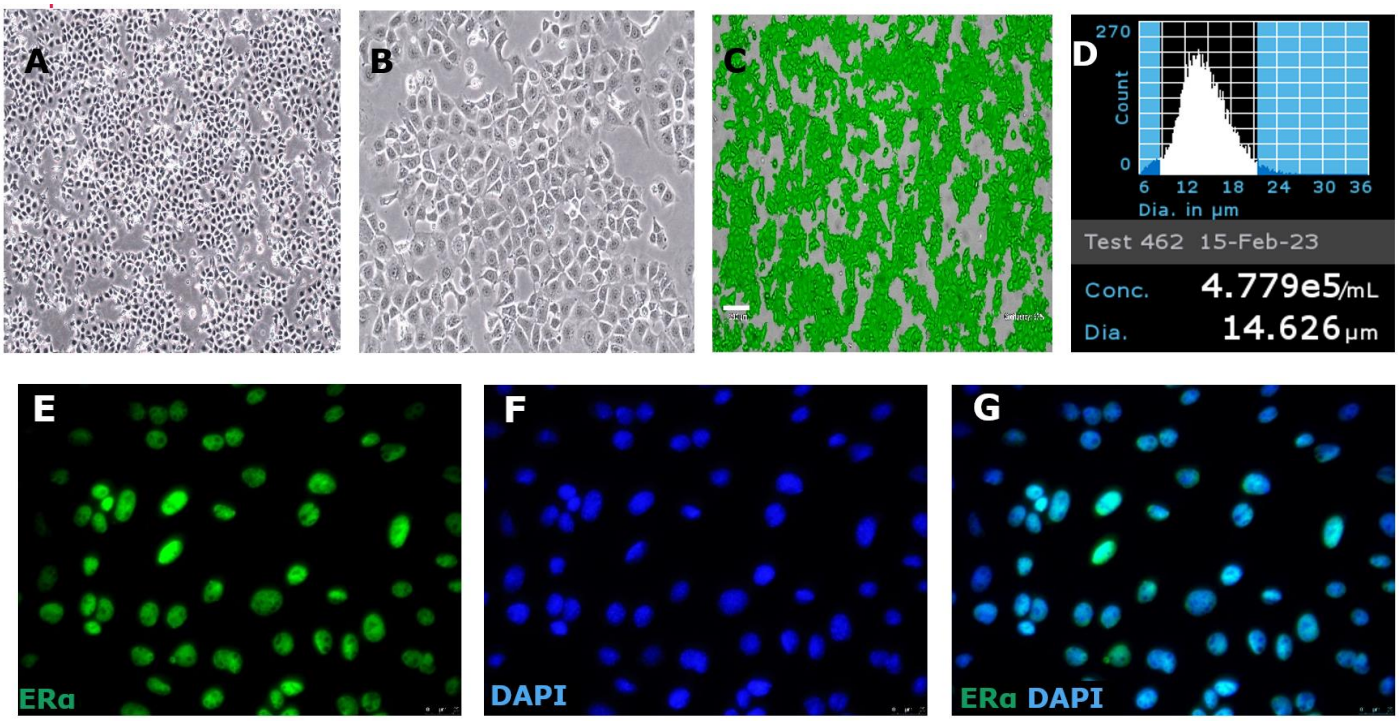

Figure 1. (4X and 10X magnification, **A**, **B**) Brightfield images of PyMT-1099 cells a day after thaw in a T75 flask. (**C**, Cat. No. MDCI10000) Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager. (**D**, Cat. No. PHCC360KIT). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 µm sensors 40X images of PyMT-1099 cells expressing Estrogen Receptor α in their nuclei (**E**, Cat. No. 06-935), Nuclei stained with DAPI (**F**, Cat. No. MBD0015) and merged image (**G**).

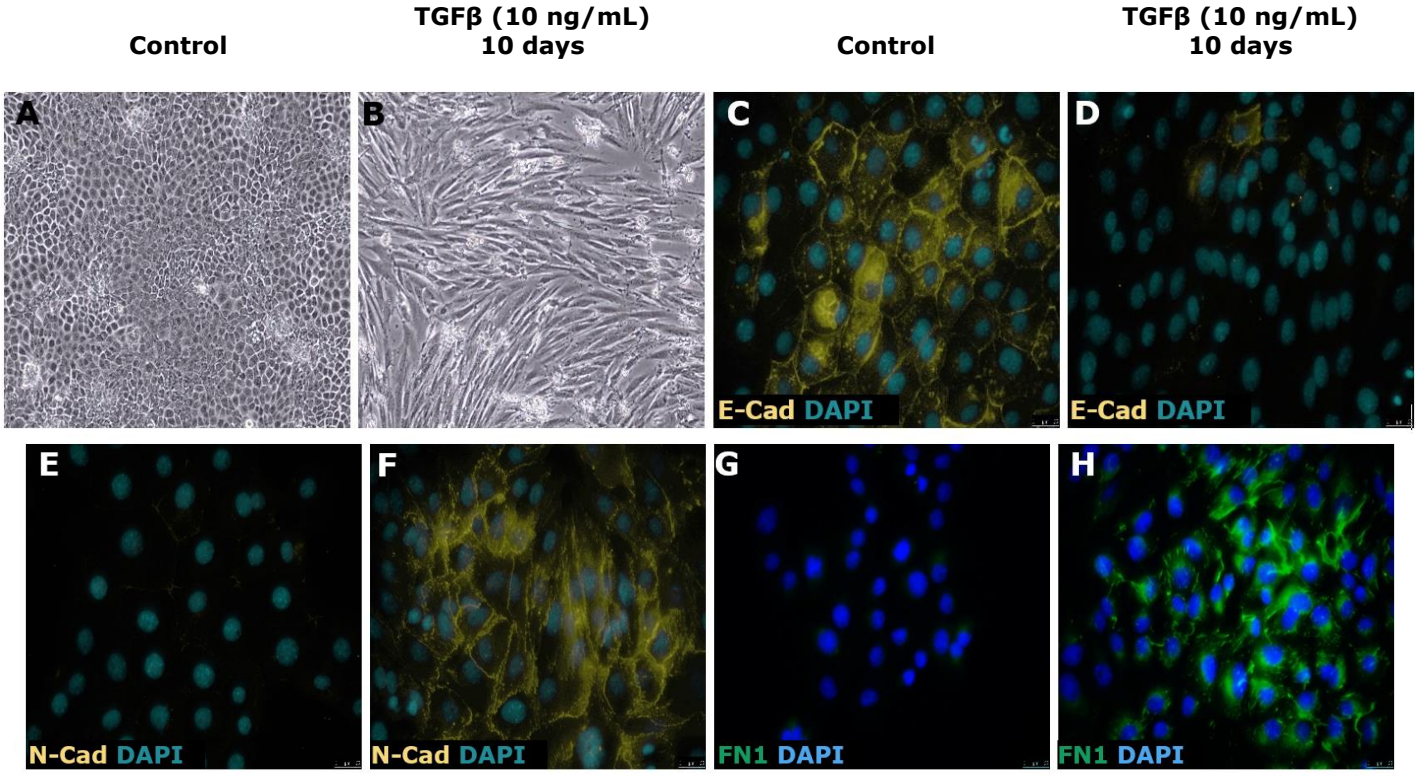

Figure 2. PyMT-1099 cells undergo epithelial mesenchymal transition upon treatment with TGFβ (10 ng/mL in expansion medium) for 10 days. 10X phase contrast image of control (**A**) and TGFβ treated(**B**) cells. Control cells express epithelial marker E-Cadherin (**C**, Fisher Scientific 13-1900) which is reduced in TGFβ treated cells (**D**). Also, TGFβ treated PyMT-1099 cells express mesenchymal markers, N-Cadherin (**F**, Cat. No. ZRB1126) and Fibronectin 1 (**H**, Cat. No. AB2033) which are absent in control PyMT-1099 cells (**E** and **G**). Images C-H are in 40X magnification.

Protocols

Either Accutase® or trypsin can be used for detaching the cells while subculturing.

Thawing the Cells

1. Do not thaw the cells until the recommended medium is on hand. Cells can grow on standard tissue cultureware surfaces without any additional coating.

   PyMT-1099 cells are thawed and expanded in PyMT-1099 Expansion Medium comprising of DMEM (Cat. No. D5796) containing 10% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. G8541) and Penicillin/Streptomycin (Cat. No. P4333), optional.
2. Remove the vial of frozen PyMT 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 PYMT-1099 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 PyMT-1099 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. PyMT-1099 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 PyMT-1099 cells.
3. Rinse the flask with 6 mL 1X sterile PBS (Cat. No. TMS-012-A). Aspirate after the rinse.
4. Apply 5-7 mL of pre-warmed Accutase® (Cat. No. A6964) and incubate in a 37 °C incubator for 5-7 minutes. Alternatively, you can use 0.25% Trypsin-EDTA pre warmed to 37 °C (Cat. No. SM-2003-C).
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 5-7 mL of PyMT-1099 Expansion Medium to the plate.
7. Gently rotate the flask to mix the cell suspension. Transfer the dissociated cells to a 15 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 PyMT-1099 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 1:6.

Cryopreservation of the Cells

PyMT-1099 cells may be frozen in PyMT-1099 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty™ container.

References

1. Shanzer M, Ricardo-Lax I, Keshet R, Reuven N, Shaul Y. 2015. The polyomavirus middle T-antigen oncogene activates the Hippo pathway tumor suppressor Lats in a Src-dependent manner. Oncogene. 34(32): 4190-4198.
2. Saxena M, Kalathur RKR, Neutzner M, Christofori G. 2018. PyMT-1099, a versatile murine cell model for EMT in breast cancer. Sci Rep. 8(1): 12123.
3. Giaquinto AN, Sung H, Miller KD, Kramer JL, Newman LA, Minihan A, Jemal A, Siegel RL. 2022. Breast Cancer Statistics, 2022. CA Cancer J Clin. 72(6): 524-541.
4. Waldmeier L, Meyer-Schaller N, Diepenbruck M, Christofori G. 2012. Py2T murine breast cancer cells, a versatile model of TGFβ-induced EMT *in vitro* and *in vivo*. PLoS One. 7(11): e48651.

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