Data Sheet

sLT-RMS Spontaneous Lung-tropic RET Melanoma-sorted Mouse Cell Line

Cancer Cell Line

SCC464

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 mouse spontaneous lung-tropic Ret-melanoma sorted (sLT-RMS) cell line is a derivative of the Ret-melanoma sorted cell line (RMS, Cat. No. SCC462). The RMS parental cells were isolated from a spontaneously occurring skin tumor in Ret transgenic mice. The Ret oncogene was originally created by an accidental *in vivo* recombination of two human genes, Ret and RFP (the Ret-finger protein, known today as TRIM27), during a transformation of the NIH3T3 cells with human lymphoma DNA.[1] The founder Ret transgenic mouse strains were generated by the embryonic microinjection of the RFP-Ret oncogene under the mouse metallothionein-I promoter-enhancer[2], and a spontaneous metastatic melanoma model line was established by repetitive rounds of back-crossing of one of such founders.[3] The parental mouse Ret-melanoma sorted (RMS) cell line (Cat. No. SCC462), was created by engineering the isolated Ret-melanoma cells to express the fluorescent reporter gene mCherry by retroviral transduction and selecting for its high expression by FACS.[4]

The mouse spontaneous lung-tropic Ret-melanoma (sLT-RMS) cell line was isolated from the lung metastasis following the subdermal injection of RMS cells.[5] Like the parental RMS line, sLT-RMS cells express mCherry and are puromycin resistant. sLT-RMS cells express the common melanoma markers PMEL and Melan-A, along with the chemokine receptor CXCR3. While CXCR3 is thought to be crucial for the ability to metastasize to the brain, sLT-RMS cells do not form brain macrometastasis upon subdermal injection. The lung-tropism of sLT-RMS appears to be organ-specific.

Source

sLT-RMS cells were derived from the lung tumors that formed upon the subdermal injection of the Ret-melanoma sorted cells (RMS), which in turn were derived from the spontaneous metastatic skin tumors in the Ret-transgenic mice. The founder transgenic mice were created by microinjection of the Ret transgene into the fertilized egg of (C57BL/6 x BALB/c) x BALB/c mice.[2] One of such founder mice was crossed with BCF1 (BALB/c x C57BL/6) mice to establish a transgenic line, designated "the 304 line." When these F1 transgenic mice were subsequently crossed with C57BL/6 mice (coat color: black), BCF1 mice (agouti), and BALB/c mice (albino), benign melanocytic tumors often developed spontaneously in the pigmented F2 offspring but did not progress to metastatic tumors.[2] The spontaneous metastatic melanoma model (designated the 304/B6 line), in which the parent of the RMS cells arose, was generated by 10 rounds of back-crossing of the 304 line with C57BL/6 mice.[3]

Short Tandem Repeat

M18-3: 15,16	M1-2: 20	M8-1: 16,17	M11-2: 16,17	MX-1: 28
M4-2: 20.3,21.3	M7-1: 26.2	M2-1: 16	M17-2: 15,16,17	M13-1: 17,18
M6-7: 15	M1-1: 17,18	M15-3: 22.3,23.3	M12-1: 17,18	
M19-2: 13	M3-2: 14,15	M6-4: 18,19	M5-5: 16,19	

Quality Control Testing

- sLT-RMS 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

sLT-RMS 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

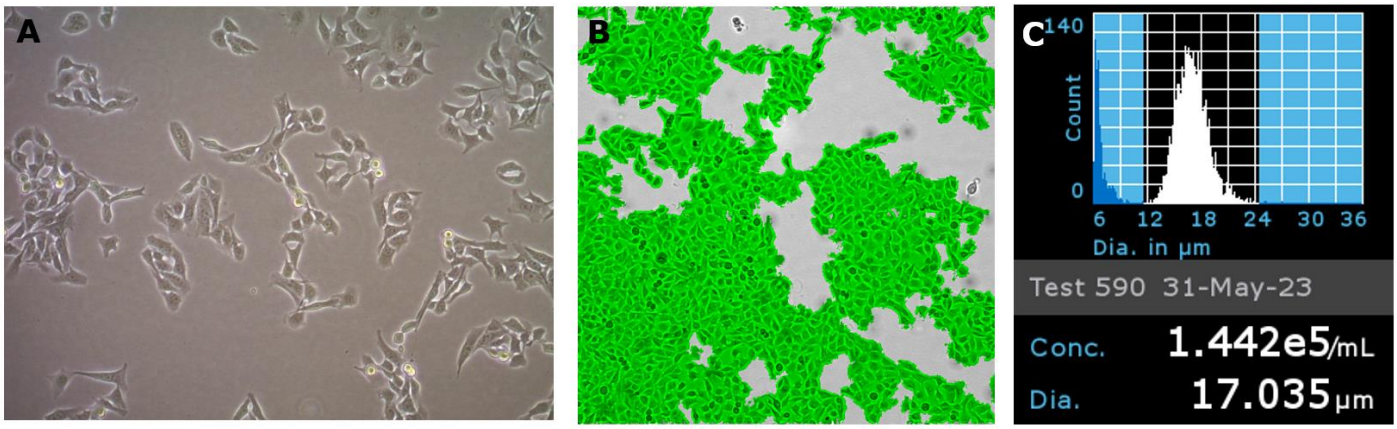

Figure 1. Bright-field image of sLT-RMS cells a day after thaw in a T175 flask (**A**). Cell confluency was assessed throughout the culture using the Millicell® Digital Cell Imager (**B**, Cat. No. MDCI10000). Cell counting was performed using Scepter™ 3.0 handheld automated cell counter using 60 µm sensors (**C**, Cat. No. PHCC360KIT).

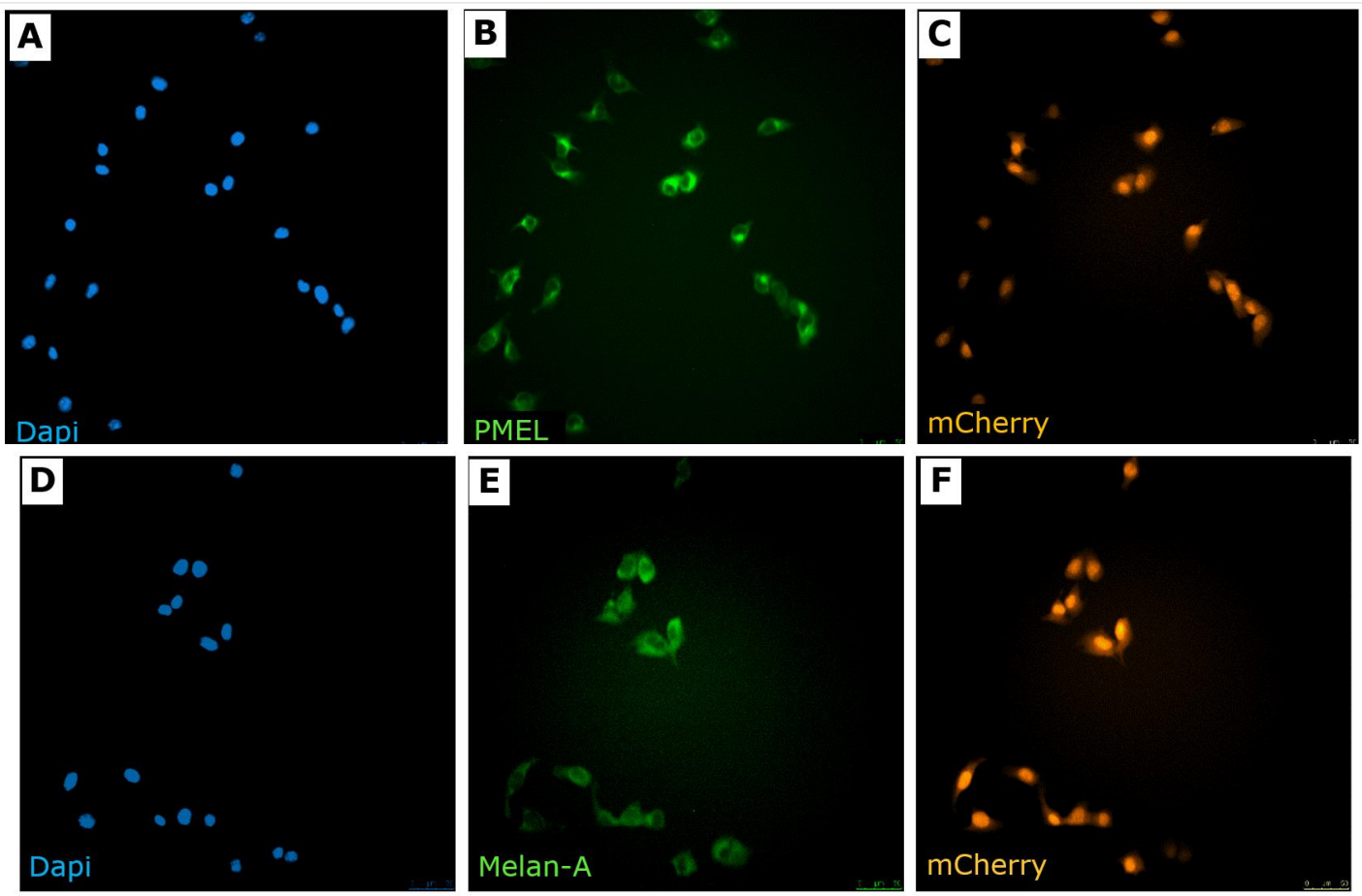

Figure 2. sLT-RMS cells express mCherry and the melanoma markers, PMEL and Melan-A. sLT-RMS cells labeled with DAPI (**A**, **D**, Cat. No. MBD0015), anti-PMEL antibody (**B**, Thermo Fisher PA5101023), anti-Melan-A antibody (**E**, Thermo Fisher PA599174), and imaged for mCherry expression (**C**, **F**).

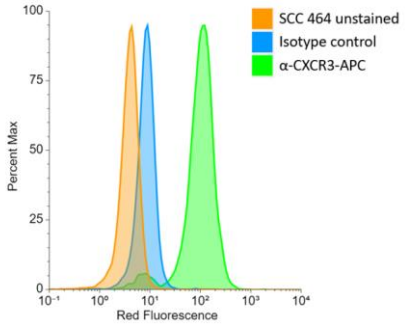

Figure 3. Analysis of the CXCR3 expression in sLT-RMS cells. Cells were either unstained (orange), stained with anti-CXCR3-APC antibodies (green, R&D Systems® FAB1685A), or stained with the isotype control antibody (blue, R&D Systems® IC006A). Histograms were obtained by Guava™ 3HT flow cytometer.

Protocols

sLT-RMS cells proliferate rapidly. We recommend thawing in a T175 flask. Either Accutase® or trypsin can be used 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 cultureware surfaces without any additional coating.

   sLT-RMS cells are thawed and expanded in sLT-RMS Expansion Medium comprising of RPMI 1640 (Cat. No. R0883) containing 10% FBS (Cat. No. ES-009-B), 2 mM L-Glutamine (Cat. No. G8541), 1 mM sodium pyruvate (Cat. No. S8636), and 2 µg/mL puromycin (Cat. No. P4512), with optional Penicillin/Streptomycin (Cat. No. 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 sLT-RMS 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 sLT-RMS 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. sLT-RMS 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 (Cat. No. TMS-012-A). Aspirate after the rinse.
4. Apply 10 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 10-15 mL of sLT-RMS Expansion Medium to the plate.
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 sLT-RMS 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

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

References

1. Takahashi M, Ritz J, Cooper GM. 1985. Activation of a novel human transforming gene, ret, by DNA rearrangement. Cell. 42(2): 581-588.
2. Iwamoto T, Takahashi M, Ito M, Hamatani K, Ohbayashi M, Wajjwalku W, Isobe K, Nakashima I. 1991. Aberrant melanogenesis and melanocytic tumour development in transgenic mice that carry a metallothionein/ret fusion gene. EMBO J. 10(11): 3167-3175.
3. Kato M, Takahashi M, Akhand AA, Liu W, Dai Y, Shimizu S, Iwamoto T, Suzuki H, Nakashima I. 1998. Transgenic mouse model for skin malignant melanoma. Oncogene. 17(14): 1885-1888.
4. Schwartz H, Blacher E, Amer M, Livneh N, Abramovitz L, Klein A, Ben-Shushan D, Soffer S, Blazquez R, Barrantes-Freer A, et al. 2016. Incipient melanoma brain metastases instigate astrogliosis and neuroinflammation. Cancer Res. 76(15):4359-4371.
5. Doron H, Amer M, Ershaid N, Blazquez R, Shani O, Lahav TG, Cohen N, Adler O, Hakim Z, Pozzi S, et al. 2019. Inflammatory activation of astrocytes facilitates melanoma brain tropism via the CXCL10-CXCR3 signaling axis. Cell Rep. 28(7): 1785-1798.

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