Data Sheet

TB096 Human IDH1-Mutated Astrocytoma Cell Line

Cancer Cell Line

SCC431

Pack Size ≥ 1x10^6 viable cells/vial

Store at: Liquid nitrogen

FOR RESEARCH USE ONLY

Not for use in diagnostic procedures. Not for Human or Animal Consumption.

Background

Astrocytoma is a cancer of the CNS (central nervous system) that can arise in the brain or spinal cord. It begins in astrocytes, a type of glial cell which performs a variety of support functions for the nervous tissue such as nutrient transport, maintenance of ionic balance, and repair processes for brain and spinal cord injury. Astrocytomas are variable and can cause a range of symptoms depending on the location of the tumor. Growth rates in astrocytomas differ significantly, ranging from slow-growing tumors to aggressive proliferation.

In glioma cells, a specific point mutation occurs with relative frequency at the Arg132 location of the IDH1 protein (NADP+-dependent isocitrate dehydrogenase 1), resulting in a gain-of-function mutation that produces D-2-hydroxyglutarate (D-2HG), a prevalent metabolite attributed to carcinogenesis and tumor progression.[1] The TB096 cell line provides a robust *in vitro* model to elucidate the role of IDH1 mutation in astrocytoma growth mediated by D-2HG conversion. This model also provides a tool for the development of therapy solutions which may modulate D-2HG production, while providing a means for studying the role of wild type IDH1 in conjunction with its mutated allele.

Source

TB096 was derived from a 26-year-old male patient treated at Duke University in 2009 for anaplastic astrocytoma WHO grade III.[1] Tumors were obtained from the Brain Tumor Biorepository at Duke with written informed consent from patients and with Institutional Review Board approval and analyzed previously for IDH mutation status.[1]

Short Tandem Repeat

D3S1358: 15	D13S317: 8, 14
D7S820: 11, 13	D16S539: 11
vWA: 15, 17	TH01: 8, 9.3
FGA: 22, 22.2	TPOX: 11
D8S1179: 12, 14	CSF1PO: 10, 12
D21S11: 30	Amelogenin: X, Y
D18S51: 16	Penta D: 14, 15
D5S818: 13	Penta E: 11, 14

Cancer cell lines are inherently genetically unstable. Instability may arise in the form of loss of heterozygosity of alleles at one or more genetic sites with increased passages.

Quality Control Testing

- TB096 cells are verified to be of human origin and negative for mouse, 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 Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells tested negative for mycoplasma.

Storage and Handling

TB096 human IDH1-mutated astrocytoma 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

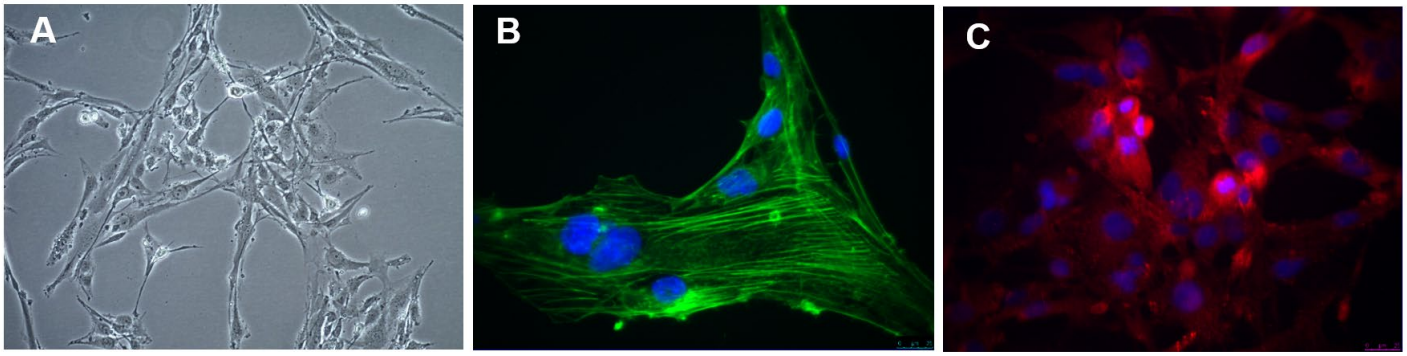

Figure 1. Bright-field images of TB096-2 cells (**A**). Cells stain for actin (green, Cat. No. P5282) and EGFR (red, Cat. No. 05-104). Blue, DAPI.

Protocols

1. Prepare TB096 Expansion Medium (250 mL):

Component	Quantity	Final Conc.	Supplier	Cat. No
Neurocult™ NS-A Proliferation Kit	110 mL	45%	StemCell Technologies	05751
DMEM-High Glucose Medium	110 mL	45%		SLM-120-B
0.2% Heparin Solution (2 mg/mL)	250 mL	2 mg/mL	StemCell Technologies	07980
EGF, 100 mg/mL stock	50 mL	20 ng/mL		01-107
FGF-2, 100 mg/mL stock	25 mL	10 ng/mL		GF003AF-100UG
Fetal Bovine Serum	25 mL	10%		ES-009-B
Ala-Gln, 200 mM Solution (100X)	2.5 mL	2 mM		G8541-100mL
Antibiotic-Antimycotic Solution (100X) (optional)	2.5 mL	1X		A5955

2. Remove the vial of frozen TB096-2 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 9 mL of TB096-2 Expansion Medium (Step 1 above) 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 2-3 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 15 mL of TB096 Expansion Medium.
10. Transfer the cell mixture to a T75 tissue culture flask.
11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

Subculturing the Cells

1. Do not allow the cells to grow to confluency. TB096-2 cells should be passaged at ~70-80% confluency.
2. Carefully remove the medium from the T75 tissue culture flask containing the 80% confluent layer of TB096 cells.
3. Rinse the flask with 10 mL 1X PBS. Aspirate after the rinse.
4. Apply 5-7 mL of Accutase™ and incubate in a 37 °C incubator for 3-5 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 5-7 mL of TB096-2 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 3-5 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 TB096-2 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.
12. Plate the cells to the desired density. Typical split ratio is 1:6.

Cryopreservation of the Cells

TB096 human IDH1-mutated astrocytoma cells may be frozen in TB096 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. Cancer Res 2013; 73(2): 496-501.

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Document Template 20306518 Ver 6.0

20674942 Ver 2.0, Rev 26MAY2023, LJ