Data Sheet

BCH869 Human H3K27M Glioma Cell Line

Tumor Cell Line

SCC218

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

Pediatric high-grade glioma (pHGG) and diffuse intrinsic pontine glioma (DIPG) are among the most aggressive and recalcitrant cancers, with extremely low survival rates. Diffuse midline gliomas (DMG) with histone H3 lysine27-to-methionine mutations (H3K27M glioma) represent a highly aggressive subtype of glioma, which predominantly arise in children and young adults. The overall prognosis of H3K27M glioma is poor, displaying median survival rates of approximately 9 to 11 months.[2] Aberrant activation of the PI3K pathway via *PTEN* silencing is a contributor to tumorigenesis in most pHG and DIP gliomas.

The BCH869 H3K27M glioma cell line is a representative model for H3K27M diffuse glioma. BCH869 cells grow as neurospheres and carry mutations in oncogenes *ACVR1, PIK3CA, and PPM1D* in addition to H3K27M mutation.[3] BCH869 cells express EGFR, a common glioma marker, and have been validated via staining for the H3.3 K27M oncohistone mutant. BCH869 cell line has been used in the investigation of radiosensitization factors and tumorigenesis pathways and is a highly clinically relevant model for this especially serious form of pediatric glioma.

Source

BCH869 cell line was derived from Pons tissue of a 7-year-old female patient.[4]

Short Tandem Repeat (STR Profile)

D3S1358:	16, 18	D18S51:	16, 19	CSF1PO:	10
D7S820:	10, 11	D5S818:	9, 11	Amel:	X
vWA:	16, 17	D13S317:	12, 14	Penta D:	12, 13
FGA:	23, 24	D16S539:	11, 13	Penta E:	7, 12
D8S1179:	11, 12	TH01:	6, 7		
D21S11:	30, 30.2	TPOX:	8		

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

- Each vial contains ≥ $1X10^6$ viable cells.
- Cells are tested negative for infectious diseases by a Human Essential CLEAR panel by Charles River Animal Diagnostic Services.
- Cells are verified to be of human origin and negative for inter-species contamination from mouse, rat, Chinese hamster, Golden Syrian hamster, and Non-human Primate (NHP) as assessed by a Contamination Clear panel by Charles River Animal Diagnostic Services.
- Cells are negative for mycoplasma contamination.

Storage and Handling

BCH869 Human H3K27M glioma cells should be stored in liquid nitrogen. The cells can be cultured for at least 10 passages after initial thawing without significantly affecting functionality.

Representative Data

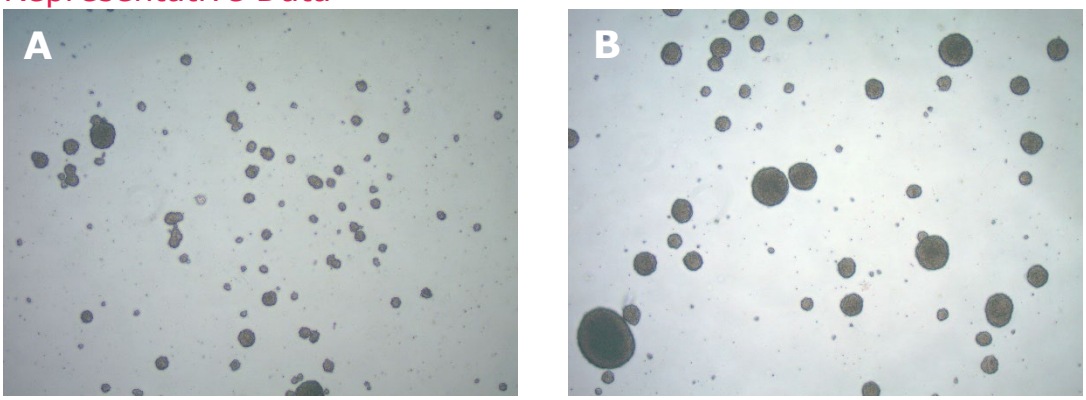

Figure 1. Bright-field images of BCH869 Human H3K27M glioma cells in culture, three (A) and seven (B) days after thaw in an ultra-low attachment T75 flask.

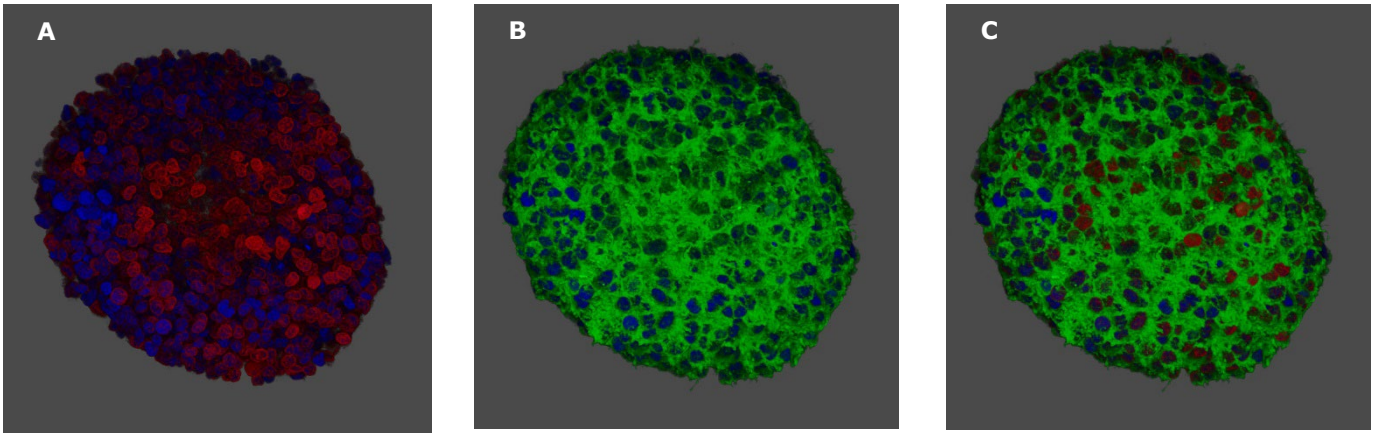

Figure 2. 3D images of BCH869 Human H3K27M glioma cells. Cells express phosphatidylinositol 3-kinase (PIK3CA) (A, red) and EGFR (B, green). Merged images (C).

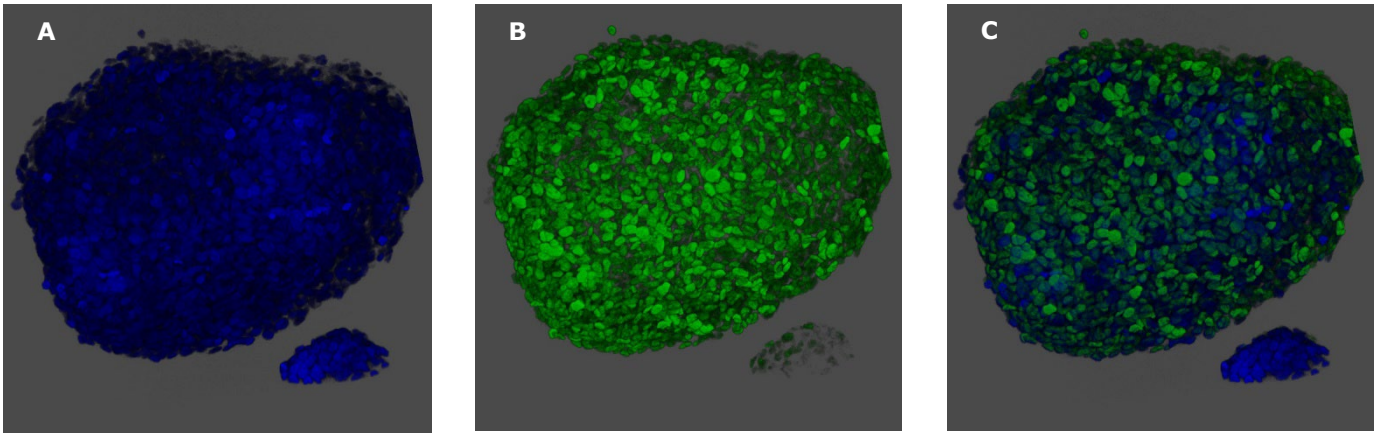

Figure 3. 3D images of BCH869 Human H3K27M glioma cells. Cells express H3K27M (B, green). Nuclei were counterstained with Dapi (A, blue). Merge images (C).

Protocols

Note: BCH869 Human H3K27M glioma cells grow as neurospheres and should be cultured on ultra-low attachment T75 flasks (Corning #3814). The cultures should be fed twice a week with growth medium that is made fresh each week. Neurospheres should be passaged every 7-8 days by dissociating into single cells by mechanical dissociation (trituration). Do not use trypsin to dissociate the cells as this will result in viability loss and should thus be avoided.

1. Prepare BCH869 Expansion Medium (100 mL). Products can be purchased at SigmaAldrich.com unless otherwise noted below.

Component	Quantity	Final Concentration	Catalog Number
Neurobasal™-A Medium (1X)	46.6 mL		10888022 (ThermoFisher®)
DMEM/F12 Medium	46.6 mL		DF-041
HEPES Buffer, 1M Solution	1 mL	10 mM	TMS-003C
MEM Sodium Pyruvate Solution (100 mM; 100X)	1 mL	1 mM	S8636
MEM, Non-Essential Amino Acids (100X)	1 mL	1X	TMS-001-C
Ala-Gln (100X)	1 mL	1X	G8541-100mL
B-27™ Supplement minus Vitamin A (50X)	2 mL	1X	12587010 (ThermoFisher®)
EGF, 20 mg/mL stock (1000X)	100 mL	20 ng/mL	01107
FGF-2, 20 mg/mL stock (1000X)	100 mL	20 ng/mL	GF003AF-100UG
Platelet Derived Growth Factor -AA (PDGF-AA, 20 mg/mL stock; 2000X)	50 mL	10 ng/mL	GF142
Platelet Derived Growth Factor -BB (PDGF-BB, 20 mg/mL stock; 2000X)	50 mL	10 ng/mL	GF149
Heparin (10,000 U/mL stock)	10 mL	1U/mL	375095-100KU
Antibiotic-Antimycotic Solution (100X); optional	1 mL	1X	A5955-100ML

2. Remove the vial of frozen BCH869 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 BCH869 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 BCH869 Expansion Medium.

10. Transfer the cell mixture to an ultra-low attachment T75 flask (3814, Corning®).

11. Incubate the cells at 37 °C in a humidified incubator with 5% CO_2.

12. Because of the instability of the media supplements and growth factors, BCH869 neurosphere culture should be fed every 2-3 days.

    For media exchanges: Transfer neurosphere suspension to a 15 mL or 50 mL conical tube. Pellet the neurospheres in a centrifuge at 1200 rpm (220 *x g*) for 5 minutes. Aspirate, leaving behind a little media. Using a 5 mL pipette, resuspend the cell pellet in 15 mL fresh medium. Pipette up and down 5X with a serological pipette. Plate cells back to ultra-low attachment T75 flask.

13. Passaging BCH869 neurospheres as single cell suspension:

    Note: Do not use Trypsin to dissociate the cells. BCH869 neurospheres grow much slower than regular monolayer cell lines. Therefore, you will need to prepare at least double the number of flasks to achieve the same number of cells as monolayer culture.

    BCH869 neurospheres should be passaged every 7-8 days. Cells are dissociated to single cell suspension by mechanical tituration. Immediately after passage, refresh media on days 3 or 4. Thereafter, exchange with fresh media every 2 to 3 days until ready to passage.

14. Collect neurospheres and centrifuge at 1200 rpm (220 *x g*) for 5 minutes. Aspirate, leaving behind a little media.

15. Using a P1000 pipette (do not use a serological pipette), resuspend the cell pellet in 600 mL expansion medium. Pipette up and down 50 times with the pipette tip touching the bottom of the conical tube to obtain a single cell suspension. Avoid the generation of air bubbles.

16. Live cell count with trypan blue: Take 5 mL of single cell suspension and resuspend in 45 mL culture medium. Add 50 mL tryphan blue (Final 20X dilution). Count number of live cells using a hemacytometer.

17. Plate approximately 6 million live cells each into 15–20 mL fresh BCH869 expansion medium in ultra-low attachment T75 flasks.

18. Exchange with fresh media on days 3 or 4 following Step 13. Thereafter, exchange with fresh media every 2-3 days until ready to passage. Cell culture growth can be assessed by the change in media color to an orange color. If the media is yellowish, this is an indication that the cell density is too high and that exchanges with fresh media are warranted along with a larger total volume of fresh media.
19. Passage every 7-8 days following Step 14 and seed approximately 6 million cells per ultra-low attachment T75 flasks.

Staining Protocol

Note: Start with high numbers of neurospheres in the T75 flask. To avoid sticking to the tube's wall, keep all solutions at 2-8 °C except for the 2nd antibody incubation.

1. Collect the suspension culture. Centrifuge at 1200 rpm (220 *x g*) for 5 minutes. Aspirate.
2. Wash the cell pellet once in 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate
3. Resuspend the cell pellet in 4% paraformaldehyde. Incubate at room temperature for 45 minites to 1 hour.
4. Wash with 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate.
5. Repeat step 4 two more times. Aspirate
6. Resuspend in 1X PBS containing 0.5% Triton™ X-100. Incubate at room temperature for 1-2 hours with shaking.
7. Centrifuge at 1200 rpm for 5 minutes. Aspirate. Directly resuspend in Cyto Q Immuno Diluent & Block (NB307, InnovexBio®) containing the antibody (1:100 dilution). Incubate at 4 °C overnight.
8. Next day, add 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate.
9. Repeat step 8 three more times.
10. Centrifuge at 1200 rpm for 5 minutes. Resuspend in Cyto Q Immuno Diluent & Block containing the 2nd antibody (1:500 dilution). Incubate at room temperature for 2 hours, then on ice for 15 minutes before centrifuging.
11. Add 1X PBS. Centrifuge at 1200 rpm for 5 minutes. Aspirate.
12. Repeat step 11 three more times. Aspirate.
13. Carefully aspirate the supernatant until the last 50-100 µL of PBS. Carefully resuspend in 200 mL DAPI working solution (Cat. No. 10236276001) and avoid causing bubbles. Transfer to 8-well chamber slide for confocal imaging. Mix the Dapi-cell suspension with Matrigel® reagent to increase the viscosity for confocal scanning.

Cryopreservation of the Cells

BCH869 Human H3K27M Glioma cells may be frozen in BCH869 Expansion Medium supplemented with 10% DMSO using a Nalgene® slow freeze Mr. Frosty® container.

References

1. *Neuro Oncol* 2017, 19(2):153-161.
2. *Front Oncol* 2020, 9:1436.
3. *Cancer Res* 2018, 78(14): 4007-4021.
4. Suva *et al.* US Patent application pub. US 2020/0384022 A1

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