NCH644

300124

GENERAL INFORMATION

Description

The NCH644 cell line is a glioblastoma stem-like cell line derived from patient tumors that lack EGFR amplification, making it a valuable model for studying glioblastoma biology, especially in the context of growth factor signaling and stem cell properties. Studies have demonstrated that in NCH644 cells, basic fibroblast growth factor (bFGF) plays a significant role in mediating growth and maintaining stem cell characteristics, whereas epidermal growth factor (EGF) does not show similar effects. NCH644 cells respond to bFGF by increasing the expression of stem cell markers such as CD133 and nestin, and they also exhibit enhanced resistance to apoptosis. This resistance, coupled with the lack of EGFR amplification, makes NCH644 a suitable model for understanding glioblastoma stem-like cell behavior, particularly under different growth factor conditions.

Another notable feature of NCH644 is its slower proliferation rate when compared to other glioblastoma stem-like cell lines, such as NCH421k. However, when stimulated by bFGF, NCH644 cells show increased expression of EGFR, even in the absence of EGFR amplification, which highlights the interaction between fibroblast growth factor receptors (FGFRs) and EGFR signaling pathways. Moreover, bFGF plays a role in increasing the clonogenicity and multipotency of NCH644 cells, further supporting the notion that bFGF is crucial for maintaining the glioma stem-like properties of these cells.

NCH644 cells have also been shown to harbor label-retaining, slow-cycling subpopulations that exhibit increased tumorigenicity and resistance to treatments such as irradiation and temozolomide. This subpopulation of label-retaining cells within the NCH644 line is highly tumorigenic, capable of forming tumors in immunocompromised mice even with small cell numbers. These features, combined with their resistance to standard treatments, make NCH644 a critical tool for investigating therapeutic strategies targeting glioblastoma stem cells.

Tissue	Brain
Disease	Glioblastoma

CLASSIFICATION

Organism	Human
Sex	Female
Biosafety level	1

CHARACTERISTICS

Tumourigenic	Yes
Ploidy status	Aneuploid

IDENTIFIERS

Citation

NCH644 (Cytion catalog number 300124)

NCBI TaxID	9606
Cellosaurus	CVCL_x914

EXPRESSION AND SUSCEPTIBILITY

Antigen expression

Highly CD133 positive

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HANDLING AND CULTURE

Culture medium

DMEM:Ham's F12 (1:1), w: 3.1 g/L Glucose, w: 2.5 mM L-Glutamine, w: 15 mM HEPES, w: 0.5 mM Sodium pyruvate, w: 1.2 g/L NaHCO3 (Cytion article number 820400a)

Incubation atmosphere	37°C, 5% CO_2, humidified atmosphere.

Supplements

Supplement the medium with 10% FBS, 5 mg/L Heparin, 20 ng/mL bFGF, 20 microgram/L EGF, 5 mg/L Insulin, 100 mg/L Transferrin, 5,2 microgram/L Na-selenit, 6,3 microgram/L Progesteron, 161,1 microgram/L Putrescin, 50 mg/L Hydrocortinson

Subculturing

For subculturing spheroid cultures, begin by mechanically dissociating the spheroids through pipetting up and down 5 to 10 times using an Eppendorf pipette with 1000 µl filter tips. After this, centrifuge the mixture at 300g for 5 minutes at room temperature to pellet the cells. Discard the supernatant and resuspend the cell pellet in fresh culture medium. Finally, transfer the resuspended cells into new culture vessels to promote further spheroid formation. This approach ensures efficient spheroid breakdown and readies them for continued growth in a new environment.

Seeding density	2 x 10^5 cells/ml
Fluid renewal	2 to 3 times per week
Post-thaw recovery	After thawing allow the cells to recover from the freezing process for at least 24 to 48 hours.

Thawing and culturing

Confirm that the vial remains deeply frozen upon delivery, as cells are shipped on dry ice to maintain optimal temperatures during transit.
Upon receipt, either store the cryovial immediately at temperatures below -150°C to ensure the preservation of cellular integrity, or proceed to step 3 if immediate culturing is required.
For immediate culturing, swiftly thaw the vial by immersing it in a 37°C water bath with clean water and an antimicrobial agent, agitating gently for 40-60 seconds until a small ice clump remains.
Perform all subsequent steps under sterile conditions in a flow hood, disinfecting the cryovial with 70% ethanol before opening.
Carefully open the disinfected vial and transfer the cell suspension into a 15 ml centrifuge tube containing 8 ml of room-temperature culture medium, mixing gently.
Centrifuge the mixture at 300 x g for 3 minutes to separate the cells and carefully discard the supernatant containing residual freezing medium.
Gently resuspend the cell pellet in 10 ml of fresh culture medium. For adherent cells, divide the suspension between two T25 culture flasks; for suspension cultures, transfer all the medium into one T25 flask to promote effective cell interaction and growth.
Adhere to established subculture protocols for continued growth and maintenance of the cell line, ensuring reliable experimental outcomes.

QUALITY CONTROL AND CRYOPRESERVATION

Sterility

Mycoplasma contamination is excluded using both PCR-based assays and luminescence-based mycoplasma detection methods.
To ensure there is no bacterial, fungal, or yeast contamination, cell cultures are subjected to daily visual inspections.

Freeze medium	As a cryopreservation medium, we use 50% basal medium + 40% FBS + 10% DMSO, or CM-1 (Cytion catalog number 800100), which includes optimized osmoprotectants and metabolic stabilizers to enhance recovery and reduce cryo-induced stress.

Storage conditions

For long-term preservation, place vials in vapor-phase liquid nitrogen at about −150 to −196 °C. Storage at −80 °C is acceptable only as a short interim step before transfer to liquid nitrogen.

Shipping conditions

Cryopreserved cell lines are shipped on dry ice in validated, insulated packaging with sufficient refrigerant to maintain approximately −78 °C throughout transit. On receipt, inspect the container immediately and transfer vials without delay to appropriate storage.

DONOR

Donor age	66 years
Ethnicity	Caucasian

LEGAL AND SAFETY

Safety precautions

When planning to store a cryovial in liquid nitrogen for future thawing, it is mandatory to adhere to stringent safety measures. Appropriate protective gloves and clothing are essential, and the use of a face mask or safety goggles is required during the transfer of frozen samples to or from the liquid nitrogen tank. This is to mitigate the risk of injury from potential cryovial explosions upon removal, which can result in the projection of sharp fragments.

Warranty

At Cytion, we stand behind the quality and viability of our products.
We guarantee that our cells will be viable at the start of culture for
60 days after shipment from our facilities.
We assure that all materials conform to the specifications described in the
Certificate of Analysis and/or Product Sheet.
For full details, please refer to our
Warranty Statement.

Third-party agreements apply	Yes