Ba/F3 FGFR3-TACC3 Cell Line

Cat. No: KC-1297 Version 19040901

Ⅰ. Cell Line Information

Catalog number	KC-1297
Cell line name:	Ba/F3 FGFR3-TACC3 Cell Line
Gene ID/Accession #:	NM_000142.4
Host cell line	Mouse Ba/F3 cell line
Cell type:	Pro-B cells
Description:	Stable Ba/F3 clone expressing exogenous FGFR3-TACC3 fusion sequence
Quantity:	One vial of frozen cells ($5X10^6$ per vial)
Stability:	Stable in culture over a minimum of 10 passages
Application:	Drug screening and biological assays
Freeze medium:	70% RMPI-1640 + 20% FBS + 10% DMSO
Propagation medium:	RPMI 1640 + 10% FBS
Selection marker:	Puromycin
Morphology:	Mostly single, round (some polymorph) cells in suspension
Subculture:	Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10^5 cells/ml
Incubation:	37 °C with 5% CO_2
Storage:	Frozen in liquid nitrogen with 70% medium, 20% FBS and 10% DMSO
Doubling time:	Approximately 20 hours
Mycoplasma status:	Negative
Biosafety level:	1
Storage:	Liquid nitrogen immediately upon receiving

Ⅱ. Background

FGFR3 is a member of the fibroblast growth factor receptor family which play a role in mitogenesis and differentiation. The FGFR3 gene produces various forms of the FGFR3 protein; the location varies depending on the isoform of the FGFR3 protein. Since the different forms are found within different tissues the protein is responsible for multiple growth factor interactions. Gain of function mutations in FGFR3 inhibits chondrocyte proliferation and underlies achondroplasia and hypochondroplasia

Ba/F3 cell, a murine interleukin-3 dependent pro-B cell line, is a popular system for exploring both kinases and their

inhibitors, because some protein kinases can render the Ba/F3 cells to be depended on the activation of the kinases instead of IL-3 supplement, while their inhibitors can antagonize the kinase-dependent growth effects.

III. Cell Line Generation

Ba/F3 FGFR3-TACC3 cell Line was generated using retrovirus vector expressing human FGFR3-TACC3 fusion sequence.

IV. Characterization

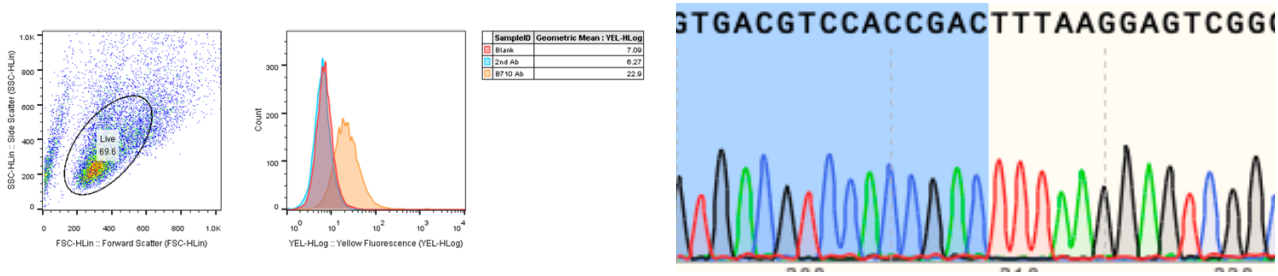


Figure: Characterization of ETV6-FGFR3-TACC3 overexpressing in Ba/F3 stable clones.

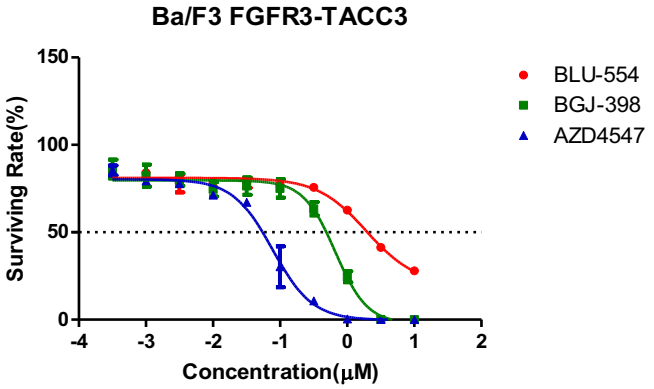
V. Application

a. Cell-based kinase inhibition screen

b. Cell viability assay

c. In vivo efficacy study

Example: kinase inhibitors screening



IC50 of FGFR3-TACC3 inhibitor

Compound	IC50 (nM)
BLU-554	2006
BGJ-398	489.02
AZD4547	54.93

1. Harvest and seed the Ba/F3 cells expressing FRFR4 mutant in 96-well plate (3000 cells/90ul medium).
2. Next day, add 10ul 10X serially diluted compound solution each well and incubate the plates for another 72 hours.
3. Add 100ul Cell Titer-Glo each well, mixed and readout using Envision.
4. Plot the dose-responsive curve and fit the IC50 (the centration of 50% inhibition of DMSO vehicle treated clones) using GraphPad Prism software (Version 5).

VI. Cell Resuscitation

1. Prewarm culture medium (RPMI-1640 supplemented with 10% FBS) in a 37°C water bath.
2. Thaw the frozen vial in a 37°C water bath for 1-2 minutes.
3. Transfer the vial into biosafety cabinet, and wipe the surface with 70% ethanol.
4. Unscrew the top of the vial and transfer the cell suspension gently into a sterile centrifuge tube containing 9.0 mL complete culture medium.
5. Spin at ~ 125 x g for 5~7 minutes at room temperature, and discard the supernatant without disturbing the pellet.
6. Resuspend cell pellet with the appropriate volume of complete medium and transfer the cell suspension into a T25 culture flask.
7. Incubate the flask at 37°C, 5% CO_2 incubator.
8. Split saturated culture 1:10 every 3 days; seed out at about 1-3 x 10^5 cells/ml.

VII. Cell Freezing

1. Prepare the freezing medium (70% RPMI-1640 + 20% FBS + 10% DMSO) fresh immediately before use.
2. Keep the freezing medium on ice and label cryovials.
3. Transfer cells to a sterile, conical centrifuge tube, and count the cells.
4. Centrifuge the cells at 250 x g for 5 minutes at room temperature and carefully aspirate off the medium.
5. Resuspend the cells at a density of at least 3 x10^6 cells/ml in chilled freezing medium.
6. Aliquot 1 ml of the cell suspension into each cryovial.
7. Freeze cells in the CoolCell freezing container overnight in a −80◦C freezer.
8. Transfer vials to liquid nitrogen for long-term storage.

VIII. References

1. "FGFR3 gene". Genetics Home Reference. U.S. National Library of Medicine. Retrieved 2018-09-27.
2. Wang Y, Liu Z, Liu Z, Zhao H, Zhou X, Cui Y, Han J (May 2013). "Advances in research on and diagnosis and treatment of achondroplasia in China". Intractable & Rare Diseases Research. 2 (2): 45–50. doi:10.5582/irdr.2013.v2.2.45

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