ETV6-NTRK3 [G623R]/BaF3

CBP73211

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I. Introduction

Cell Line Name: ETV6-NTRK3 [G623R]/BaF3

Host Cell：Ba/F3

Stability: 16 passages (in-house test, that not means the cell line will be instable beyond the passages we tested.)

Application: Anti-proliferation assay and PD assay

Freeze Medium: 90% FBS+10% DMSO

Complete Culture Medium: RPMI-1640+10%FBS+2ug/ml puromycin

Mycoplasma Status: Negative

II. Background

ETV6-NTRK3 gene fusion is the translocation of genetic material between the ETV6 gene located on the short arm (designated p) of chromosome 12 at position p13.2 (i.e. 12p13.2) and the NTRK3 gene located on the long arm (designated q) of chromosome 15 at position q25.3 (i.e. 15q25.3) to create the (12;15)(p13;q25) fusion gene, ETV6-NTRK3. This new gene consists of the 5' end of ETV6 fused to the 3' end of NTRK3. ETV6-NTRK3 therefore codes for an chimeric oncoprotein consisting of the helix-loop-helix (HLH) protein dimerization domain of the ETV6 protein fused to the tyrosine kinase (i.e. PTK) domain of the NTRK3 protein. The ETV6 gene codes for the transcription factor protein, ETV6, which suppresses the expression of, and thereby regulates, various genes that in mice are required for normal

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hematopoiesis as well as the development and maintenance of the vascular network. NTRK3 codes for Tropomyosin receptor kinase C a NT-3 growth factor receptor cell surface protein that when bound to its growth factor ligand, neurotrophin-3, becomes an active tyrosine kinase that phosphorylates tyrosine residues on, and thereby stimulates, signaling proteins that promote the growth, survival, and proliferation of their parent cells. The tyrosine kinase of the ETV6-NTRK3 fusion protein is dysfunctional in that it is continuously active in phophorylating tyrosine residues on, and thereby continuously stimulating, proteins that promote the growth, survival, and proliferation of their parent cells. In consequence, these cells take on malignant characteristics and are on the pathway of becoming cancerous. Indeed, the ETV6-NTRK3 fusion gene appears to be a critical driver of several types of cancers. It was originally identified in congenital fibrosarcoma and subsequently found in secretory breast cancer (also termed juvenile breast cancer), Mammary analogue secretory carcinoma of salivary glands (also termed MASC or MASCSG), congenital fibrosarcoma, congenital mesoblastic nephroma, rare cases of acute myelogenous leukemia, ALK-negative Inflammatory myofibroblastic tumour, and radiation-induced papillary thyroid carcinoma.

III. Representative Data

1.Sanger of ETV6-NTRK3 [G623R]/BaF3

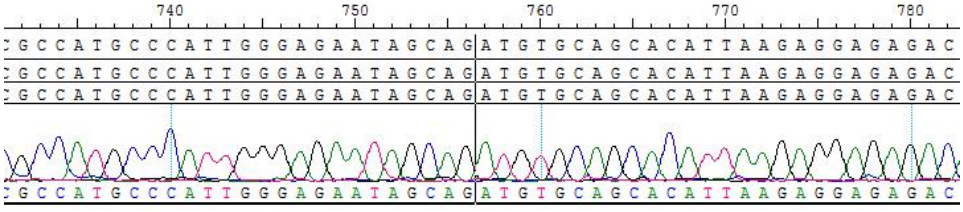

Figure 1. Breakpoint of ETV6-NTRK3-G623R/BaF3

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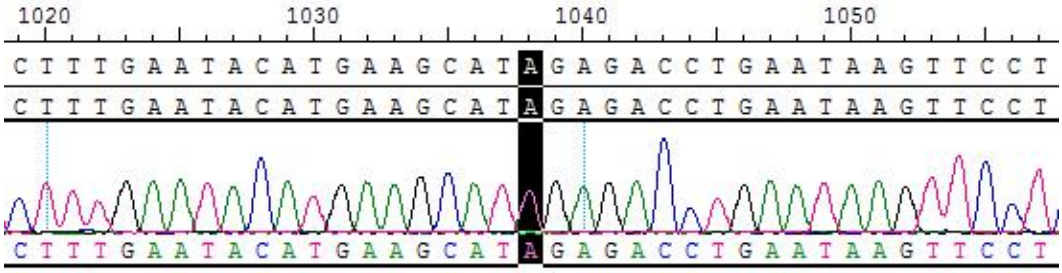
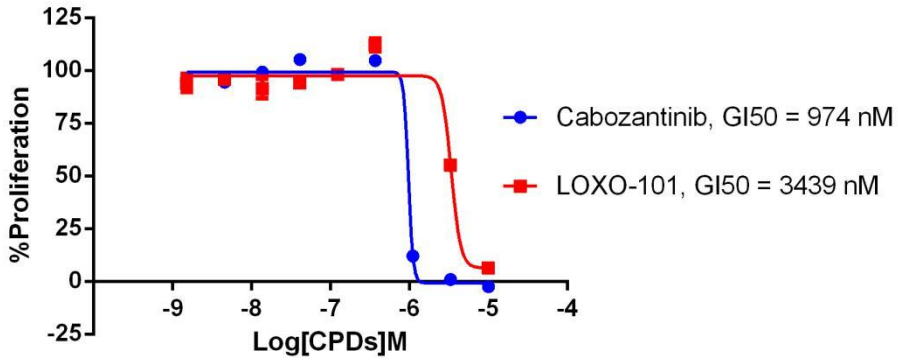


Figure 2. SNP of ETV6-NTRK3-G623R/BaF3

2. Anti-proliferation assay



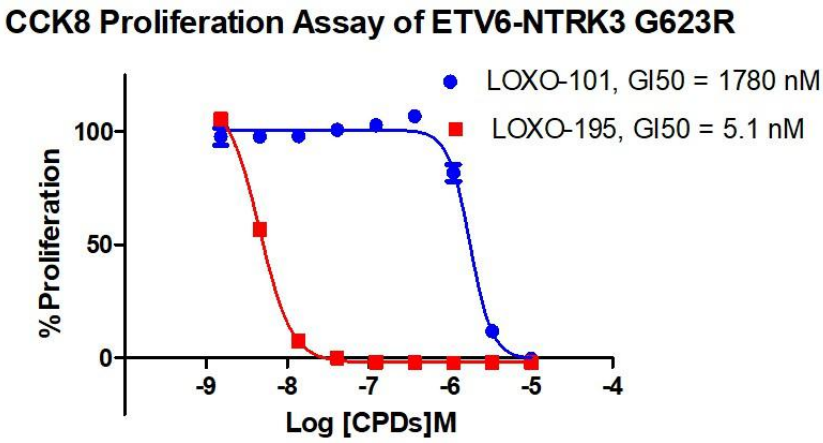


Figure 3. Anti-proliferation assay of two reference compounds on the ETV6-NTRK3-G623R/BaF3 Stable Cell Line.

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IV. Handling Procedure for Flask Cultures

The flask was seeded with cells grown and completely filled with medium at Cobioer.

1. Upon receipt visually examine the culture for macroscopic evidence of any microbial contamination.

Using an inverted microscope (preferably equipped with phasecontrast optics), carefully check for any evidence of microbial contamination.

2. Incubate the flask in an upright position for several hours at 37°C. After the temperature has equilibrated, aseptically remove the entire contents of the flask and centrifuge at 1000 rpm for 5 minutes.Remove shipping medium and save for reuse.Resuspend the cell pellet in 10 ml of this medium.

3. From this cell suspension remove a sample for a cell count and viability.Adjust the cell density of the suspension to $3*10^5$viable cells/ml in the shipping medium.

4. Incubate the culture, horizontally at 37°C in a 5% CO_2 in air atmosphere. Maintain the cell density of the culture as suggested under the subculture procedure.

V. Subculturing Procedure

Cultures can be maintained by the addition of fresh medium or replacement of medium. Alternatively, cultures can be established by centrifugation with subsequent resuspension at $3*10^5$ viable cells/mL. Do not allow the cell density to exceed $2*10^6$ cells/mL.

Interval: Maintain cultures at a cell concentration between $3*10^5$ and $2*10^6$ viable cells/mL.

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Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)

VI. Cryopreservation Procedure

1.Transfer cell suspension(4-8*10^6 cells) to a centrifuge tube and spin at approximately 1000rpm for 5 minutes.

2. Discard supernatant and resuspend cells in cryopreservation medium(90% FBS+10%DMSO).

3. Transfer the cells into Freezing Tube, 1ml/vial.

4. Place Freezing Tube in a Frosty container that is kept at room temperature and has sufficient isopropanol.

5. Place the Frosty container into the -80°C freezer overnight.

6. On next day, transfer the vials into liquid nitrogen.

VII. Performing the Anti-proliferation Assay

1. Materials to Be Supplied by the User

- Compounds to be tested
- RPMI-1640 (Gibco #C11875500CP)
- FBS (Gibco #10099-141C)
- DMSO
- CellTiter-Glo® Luminescent Cell Viability Assay (Promega, Cat.No.: G7573)
- 96 Well Assay Plate (White Plate, Clear Bottom with Lid Tissue Culture Treated Polystyrene 1/Pack, Corning #3610)
- T25 Flask
- 96 Well Storage Microplate(Corning#3357)

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- Synergy H1 Hybrid Multi-Mode Reader(Biotek)

2. Protocol for the Anti-proliferation Assay

1) Take logarithmic growth cells, centrifuge and discard the culture supernatant, resuspend the centrifuged cells in fresh RPMI-1640+10%FBS medium.

2) Inoculate the resuspended cells into a 96-well cell culture plate with a white wall and transparent bottom, 100ul/well of cell suspension, inoculate two culture plates, and place in a 37°C cell incubator for overnight culture.

3) The next day, take one of the 96-well plates inoculated with cells, add 100ul/well cell titer glo detection reagent and leave it for 60 minutes, read the value, and define it as G0 data.

4) Take another parallel plate, and take 11.1 ul/well of compound from the previously diluted 10*compound concentration dilution plate to this plate , And set up another DMSO control well, continue to incubate in a 37°C cell incubator for 72 hours.

5) Take out the 96-well plate that has been treated with the compound for 72 hours from the incubator, add 100ul/well cell titer glo detection reagent and leave it for 60 minutes, read the value, and define it as G3 data.

6) Calculate the cell proliferation rate corresponding to each well according to the following formula %Proliferation = (Test compound well G3-G0 average value) / (DMSO control well G3 average value-G0 average value)*100.

7) According to the corresponding proliferation rate and concentration of each gradient concentration hole, use Prism Graphpad5.0 software to fit the gradient curve of cell proliferation, and calculate the GI50 of the

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compound (GI50 is defined as the corresponding compound concentration when the cell proliferation rate is 50% , The fitting formula in the software is as follows: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)).

VIII. References

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