EGFR WT (EGF Dependent)/BaF3

CBP73166

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I. Introduction

Cell Line Name: EGFR WT (EGF Dependent)/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+100ng/ml EGF

Mycoplasma Status: Negative

II. Background

EGFR is widely recognized for its importance in cancer. Amplification and mutations have been shown to be driving events in many cancer types. Its role in non-small cell lung cancer, glioblastoma and basal-like breast cancers has spurred many research and drug development efforts. Tyrosine kinase inhibitors have shown efficacy in EGFR amplfied tumors, most notably gefitinib and erlotinib. Mutations in EGFR have been shown to confer resistance to these drugs, particularly the variant T790M, which has been functionally characterized as a resistance marker for both of these drugs. The later generation TKI's have seen some success in treating these resistant cases, and targeted sequencing of the EGFR locus has become a common practice in treatment of non-small cell lung cancer. Overproduction of ligands is another possible mechanism of activation of

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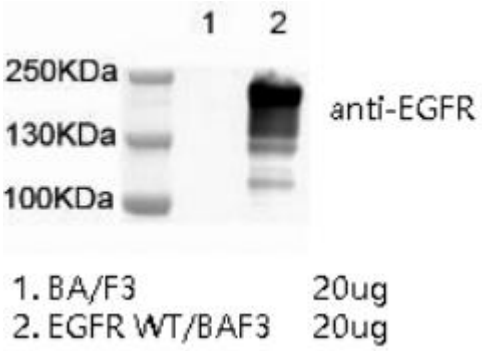
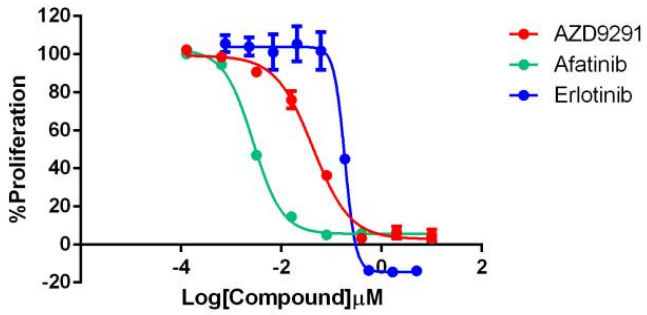
EGFR. ERBB ligands include EGF, TGF-a, AREG, EPG, BTC, HB-EGF, EPR and NRG1-4 (for detailed information please refer to the respective ligand section). In ligand-activated cancers, Cetuximab appears to be more effective than tyrosine-kinase inhibitors.

III. Representative Data

1.WB of EGFR WT (EGF Dependent)/BaF3



2. Anti-proliferation assay



Compound	Gi50(uM)
AZD9291	0.0426
Afatinib	0.0027
Erlotinib	0.186

Figure 2. Anti-proliferation assay of three reference compounds on the EGFR WT (EGF Dependent)/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. Medium Renewal: Add fresh medium every 2 to 3 days (depending on cell density)

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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)
- Synergy H1 Hybrid Multi-Mode Reader(Biotek)

2. Protocol for the Anti-proliferation Assay

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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 compound (GI50 is defined as the corresponding compound concentration when the cell proliferation rate is 50% , The fitting formula

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in the software is as follows: Y=Bottom + (Top-Bottom)/(1+10^((LogIC50-X)*HillSlope)).

VIII. References

1. Clinicopathologic features of non-small-cell lung cancer with EML4-ALK fusion gene. (PMID: 20183914) Takahashi T … Date H *Annals of surgical oncology 2010* 3 22 40

2. KRAS mutation status in primary nonsmall cell lung cancer and matched metastases. (PMID: 20336783) Cortot AB … Hainaut P *Cancer 2010* 3 22 40

3. Are there imaging characteristics associated with epidermal growth factor receptor and KRAS mutations in patients with adenocarcinoma of the lung with bronchioloalveolar features? (PMID: 20087229) Glynn C … Ginsberg MS *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 2010* 3 22 40

4. TP53, EGFR, and KRAS mutations in relation to VHL inactivation and lifestyle risk factors in renal-cell carcinoma from central and eastern Europe. (PMID: 20137853) Szymańska K … Brennan P *Cancer letters 2010* 3 22 40

5. Genetic and proteomic features associated with survival after treatment with erlotinib in first-line therapy of non-small cell lung cancer in Eastern Cooperative Oncology Group 3503. (PMID: 20035238) Amann JM … Carbone DP *Journal of thoracic oncology : official publication of the International Association for the Study of Lung Cancer 2010* 3 22 40

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