Examining the efficacy of a novel small molecule inhibitor of EGFR and ERBB2

 Parsa, Tzu-Hsiu Chen, Kumiko Tanaka, and Heidi Greulich

 Broad Institute of MIT and Harvard, Cambridge, MA 02139

Introduction

Growth factor receptors allow cells to receive growth signals from the extracellular environment, in the form of proteins such as Epidermal Growth Factor (EGF). EGFR and ERBB2 are growth factor receptor tyrosine kinases. In the presence of EGF, EGFR and ERBB2 can heterodimerize to promote cell proliferation and survival. Mutations in the genes encoding EGFR and ERBB2 are found in lung cancer. A subset of these mutations are insertion mutations that have been shown to have oncogenic properties in cultured cells.

The main goal of this project is to test the efficacy of a new drug that targets the ERBB2 pathway. We aim to compare its efficacy to that of two other drugs already on the market and one other drug that is currently in clinical trials.

The EGFR/ERBB2 signal transduction pathway

Small molecule inhibitors that target the ERBB2 pathway have been synthesized, but their clinical efficacy remains elusive. The compound of focus in our studies is DSI-1, a small molecule which is a dual-specificity EGFR and ERBB2 inhibitor. We aim to test how effective this drug is in the laboratory before it undergoes clinical testing. Our studies compare this drug to three other drugs: Erlotinib, Rapamycin, and DSI-2, a compound similar to DSI-1. DSI-2 has been shown to be effective in the laboratory but there are implications that it has not been effective in the clinic.

We are testing the efficacy of DSI-1 and other small molecule inhibitors on hematopoietic Ba/F3 cells expressing several oncogenic ERBB2 mutants. Ba/F3 cells are normally dependent on a cytokine named interleukin-3 (IL-3) for survival and proliferation. However, expression of oncogenes such as the ERBB2 mutants found in lung cancer supports proliferation of the cells in the absence of IL-3. The pink region is the area in which amino acids are inserted in the ERBB2 mutant proteins.

Results and Conclusions

Confirming the Poor Efficacy of Erlotinib

Against ERBB2 Mutant Cells

Conclusion: These data show that our assay is working as expected, with Erlotinib inhibiting cell proliferation only at high concentrations.

Comparing Efficacies of Erlotinib, DSI-1 and DSI-2

Conclusion: DSI-1 is more effective than Erlotinib, a currently used treatment. DSI-1 may not be quite as effective as DSI-2, a compound currently undergoing clinical testing.

Materials and Methods

Cell Lines Used

All cell lines used were Ba/F3 hematopoietic cells. Each cell line used overexpresses one of the following ERBB2 constructs:

- ERBB2 Ins 1
- ERBB2 Ins 2
- wild-type ERBB2

Chemical Compounds Used

- Rapamycin, a molecule named mTOR that inhibits a downstream signaling protein named in TOR
- Erlotinib, an EGFR inhibitor

Procedural Outline

- Split Ba/F3 cells and grow in IL-3 media until confluent
- Incubate cells for 12 hours
- Take cells to Coulter Counter to determine number of viable cells per mL
- Plate appropriate number of cells and grow in media lacking IL-3
- Expose cells to different concentrations of drugs (Rapamycin, Erlotinib, DSI-1, or DSI-2) for 48 hrs
- Perform WST assay by adding reagent, incubating, and reading luminescence in Molecular Devices Spectramax 190
- Analyze WST assay results to determine number of viable cells present under each experimental condition

Oncogenic ERBB2 mutations in the kinase domain

Portion of wild-type ERBB2

Portion of ERBB2 Insertion A775_G776 ins V7GA ("Ins 1")

Portion of ERBB2 Insertion A775_G776 ins YVMA ("Ins 2")

The figure shown above is a structure of the EGFR kinase domain, which is similar to the ERBB2 kinase domain. The pink region is the area in which amino acids are inserted in the ERBB2 mutant proteins.

References


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