Breast cancer tumorigenesis is a complex disease where multiple signaling pathways participate in cell proliferation and invasion. The heterogeneity of this disease presents a challenge in developing therapeutic treatments because patients respond to therapies with varying degrees of sensitivity. For example, previous studies have demonstrated that activation of the Wnt/MAPK pathway, loss of cell adhesion, and enhanced epithelial-to-mesenchymal transition confer resistance to the breast cancer therapeutic, gefitinib.

With the advent of zinc-finger nucleases (ZFN) technology, it is possible to generate cancer-relevant mutations in one or more endogenous genes. The zinc-finger nucleases have been optimized to target specific genes where they induce a double-strand break adjacent to their binding site. Double-strand breaks are either repaired by non-homologous end joining (NHEJ) or homology-directed repair (HDR). As a result, cell line harbors insertions, deletions, or integrations within the targeted gene of interest.

We have utilized zinc-finger nucleases to target and disrupt endogenous breast cancer relevant genes, SYK, ESR2, BCR, and APC, in the near normal mammary epithelial line, MCF10a. These gene targets play a pivotal role in cellular proliferation, migration and cell adhesion and may thereby play a contributing role in gefitinib sensitivity.

In this study, we aim to utilize zinc finger nuclease technology to target and disrupt endogenous breast cancer relevant genes, SYK, ESR2, BCR, and APC, in the near normal mammary epithelial line, MCF10a. These gene targets play a pivotal role in cellular proliferation, migration and cell adhesion and may thereby play a contributing role in gefitinib sensitivity.

In this study, we aim to investigate how loss of SYK, ESR2, BCR, and APC gene function, in ZFN engineered cell lines, affect sensitivity towards the tyrosine kinase inhibitors: gefitinib.

ZFN Targeting Mechanism

(A). ZFN Pair Delivered into Cell by Electroporation

(B). 20% Makes a Double Strand Break

(C). ZFN Targeting Mechanism

(ZFN Targeting Mechanism)


*Corresponding author: Gregory Wemhoff, Sigma-Aldrich Corp., 2000 Ida Ave. St. Louis, MO 63103, ph: 888-521-8956 ext.3514, e-mail: gregory.wemhoff@aldrich.com

Conclusion/Discussion

• ZFN engineered cell lines provide a model system where cancer relevant genes can be endogenously altered and used as a tool to screen therapeutic agents.

• Our data demonstrate that varying degrees of drug sensitivity can be achieved depending on the individual gene target modified. These data underscore the complexity of this disease and call to attention the importance of identifying therapeutic agents to treat patient specific cancer.

• New strategies may be developed using ZFN modified cell lines, offering enhanced efficacy in personal cancer treatments.

• Additional cell lines available for future screening include, but are not limited to, MCF10A, MCF10C, HSG, HREC, MCF7, HAPY, CCK28, AKT2, and AKT1.

Endnotes/References


*Corresponding author: Gregory Wemhoff, Sigma-Aldrich Corp., 2000 Ida Ave. St. Louis, MO 63103, ph: 888-521-8956 ext.3514, e-mail: gregory.wemhoff@aldrich.com

Where bio begins

Genetic Modification of Breast Cancer Gene Targets Using ZFN Technology Reveal Differential Responses to Drug Sensitivity

Laura Daley, Courtney Corman, Suzanne Hibbs, Gene Pegg, Andrea Spencer, Hamideh Zakeri, Zhihong Zhang, Gary Davis and Gregory Wemhoff

Cell-Based Assays, Research Biosciences, Sigma-Aldrich Corporation, 2000 Ida Ave St. Louis, MO 63103, USA

Workflow

1. Nucleate MCF10a cells with a gene targeted ZFN pair.

2. Seed 1x10^6 cells and grow to 20%.

3. Harvest at 30°C for approximately 3 weeks.

4. Harvest and sequence clones.

5. Recover at 30°C for approximately 3 days.

6. Maintain at 37°C for approximately 3 days.

7. Identify clones exhibiting out of frame insertions. Expand cells for future use.


9. Percentage viability was determined in comparison to MCF10a cells harboring wild type SYK.

10. MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type SYK.

11. Attenuated Cell Death in APC-/- Cells Following Gefitinib Exposure. (B).

12. Percentage viability was determined in comparison to MCF10a cells harboring wild type ESR2.

13. MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type ESR2.


15. Percentage viability was determined in comparison to MCF10a cells harboring wild type APC.

16. MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 100 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type APC.

17. Attenuated Cell Death in APC-/- Cells Following Gefitinib Exposure. (D).

18. Percentage viability was determined in comparison to MCF10a cells harboring wild type BCR.

19. MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type BCR.


21. Percentage viability was determined in comparison to MCF10a cells harboring wild type APC.

22. MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 100 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type APC.

23. Attenuated Cell Death in APC-/- Cells Following Gefitinib Exposure. (F).

24. Percentage viability was determined in comparison to MCF10a cells harboring wild type APC.

25. MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type APC.