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

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Introduction

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 [1,2]. For example, previous studies have demonstrated that activation of the MEK/MAPK pathway, loss of cell-cell adhesion, and enhanced epithelial-to-mesenchymal transition confer resistance to the breast cancer therapeutic, gefitinib [2,3,4].

With the advent of zinc finger nuclease (ZFN) technology, it is possible to generate cancer relevant mutations in one or more endogenous genes. 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 dependent repair (HDR). As a result, cell lines harbor insertions, deletions, or integrations within the targeted gene of interest.

We have utilized 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 [5,6,7,8,9,10]. 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 inhibitor, gefitinib.

ZFN Targeting Mechanism

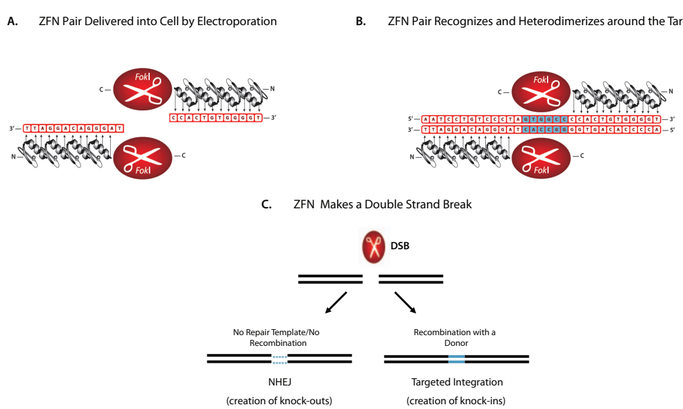
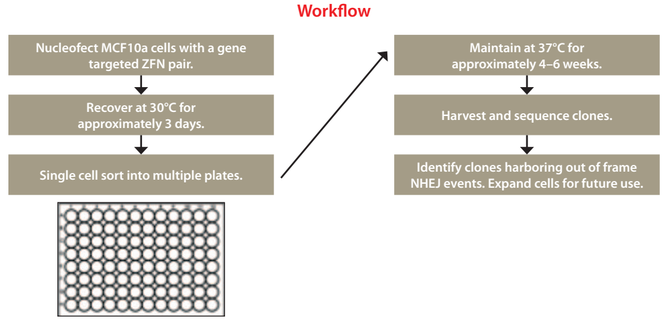


Figure 1: ZFN Targeting Mechanism. (A). ZFN-mediated genome editing takes place in the nucleus when a ZFN pair targeting the user's gene of interest is delivered into a parental cell line, either by transfection, electroporation, or viral delivery. **(B).** ZFNs bind to the target site. The Fok1 endonuclease domain dimerizes and makes a double strand break (DSB) between the binding sites. **(C).** DSBs are repaired by either an error-prone NHEJ pathway or high-fidelity homologous recombination. NHEJ introduces deletions or insertions, which change the spacing between the binding sites so that ZFNs might still bind but dimerization or cleavage cannot occur.

Workflow



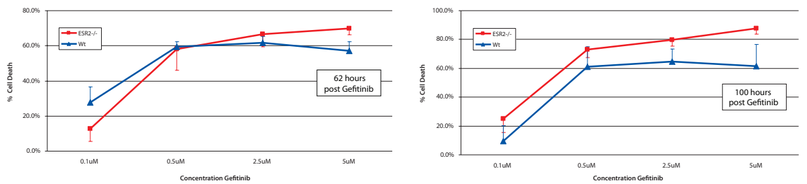


Figure 2: Augmented Cell Death in ESR-/- Cells Following Gefitinib Exposure. (A). MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in compared to MCF10a cells harboring wild type ESR2. **(B).** 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 ESR2.

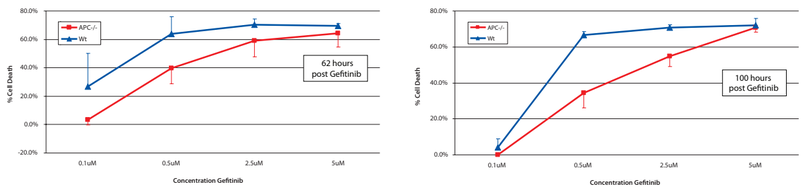


Figure 3: Attenuated Cell Death in APC-/- Cells Following Gefitinib Exposure. (A). MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type APC. **(B).** 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.

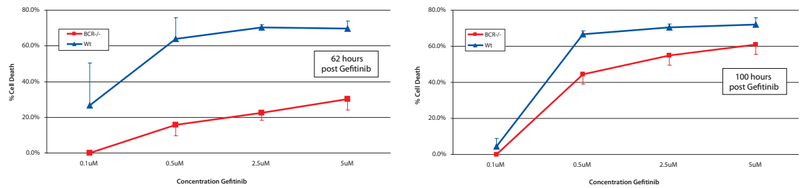


Figure 4: Attenuated Cell Death in BCR-/- Cells Following Gefitinib Exposure. (A). MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type BCR. **(B).** 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 BCR.

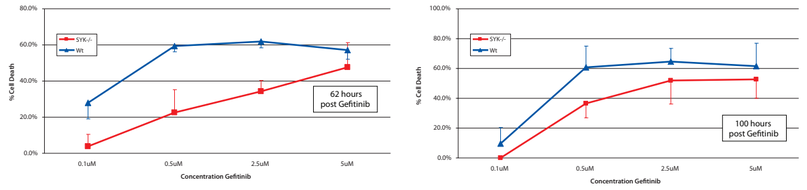


Figure 5: Attenuated Cell Death in SYK-/- Cells Following Gefitinib Exposure. (A). MCF10a cells were examined for viability using a chemiluminescent assay. Cells were exposed to the indicated doses of gefitinib and viability determined following a 62 hour incubation period. Percent viability was determined in comparison to MCF10a cells harboring wild type SYK. **(B).** 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 SYK.

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 personalized cancer treatments.
- Additional cell lines available for future screening include, but are not limited to, PTEN, EGFR, CDH1, GSK3B, HER2, TP53, EF2K, H2AFX, CDC25B, AKT2, PARP2, and AKT1.

Endnotes/References

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