Introduction

Human epidermal growth factor receptor 2 (HER2) and epidermal growth factor receptor (EGFR) are both members of the ErbB family of receptor tyrosine kinases. The study of this receptor family is of high therapeutic interest as their overexpression has been associated with numerous types of cancer as well as acquired resistance to some chemotherapeutic agents. We have developed novel genetically engineered cell lines as unique research tools to study these receptors.

Zinc finger nucleases (ZFNs) are synthetic proteins engineered to bind DNA at a sequence-specific location and create a double strand break. The cell's natural machinery repairs the break in one of two ways: non-homologous end joining (NHEJ) or homologous recombination. We utilized the homologous recombination pathway to transduce a specific location of the gene of interest, in this case, the desired location was near the C-terminus of HER2 and/ or EGFR and the transgene chosen was a fluorescent reporter protein.

In addition to endogenous tagging of specific genes, we also designed a domain-based, genetically encoded biosensor for EGFR by transduction with lentivirus. The biosensor is composed of a specific protein-binding domain, derived from known natural domain-binding proteins, fused to a fluorescent protein that serves as a reporter.

Materials and Methods

A549 and SK-OV-3 cells (Cat. No. CCL-185 and HTB-77) cells were obtained from ATCC. The SK-OV-3 cells were cultured according to the product manual and A549 were grown in RPMI-1640, 2 mM glutamine, 10% FBS in 5% CO2, 37°C.

Fluorescent reporter genes were obtained from Evrogen (http://evogen.com/products/taggFlu.sh.html). CompoZr® ZFNs were designed and manufactured by Sigma-Aldrich. Fluorescent microscopy was performed with a Nikon Eclipse TE2000-E inverted research microscope and Meta Morph® software. Unless otherwise indicated, all reagents and materials used in this work were obtained from Sigma-Aldrich (St. Louis, MO USA).

Discussion and Conclusion

Traditionally, biochemical assays and immunohistochemical staining methods have been used to study cellular pathways. We have shown two approaches that allow for live cell imaging studies of endogenous HER2 and EGFR activity: ZFN mediated tagging of endogenous fusion proteins and a genetically encoded receptor specific biosensor.

With ZFN-mediated gene tagging in knock-in cell lines, EGFR and HER2’s native gene regulation is conserved resulting in normal protein expression levels and preservation of protein function. In contrast to biochemical assays or immunostaining, using a tagged protein under endogenous regulation allows detection of RTK activation in live cells. In wild type cells, activation of EGFR leads to internalization through endocytosis. A lentiviral construct of the EGFR biosensor was used to transfect A549 cells. Single cell clones were selected and assayed for activity with EGF. When EGF is added, the biosensor migrates to the cell membrane and is subsequently internalized through endocytosis.

Current FP-tagged Cell Line Product Offerings

- Lung Carcinoma Cell Line with EGFR SH2 Biosensor – CLL1097
- Ovarian Carcinoma Cell Line with GFP-tagged HER2 – CLL1135
- Osteosarcoma Cell Line with GFP-tagged NUP98 – CLL1136
- Ovarian Carcinoma Cell Line with GFP-tagged STAT3 – CLL1139
- Lung Carcinoma Cell Line with RFP-tagged STAT3 – CLL1140
- Lung Carcinoma Cell Line with GFP-tagged EGFR – CLL1141
- Lung Carcinoma Cell Line with GFP-tagged HER2 and RFP-tagged EGFR – CLL1143

References