Introduction

The purpose of the study was to detect the effects of gefitinib (brand name Iressa by AstraZeneca Pharmaceuticals) on the phosphorylation of downstream targets of EGFR (Figure 1) and to show the application of the Bio-Plex phosphoprotein assays to drug discovery based on signal transduction pathways. Gefitinib, an inhibitor of epidermal growth factor receptor (EGFR), is used to treat non-small cell lung cancer (NSCLC). The responses of NSCLC to gefitinib are related to mutations occurring within the EGFR kinase domain. Because Bio-Plex phosphoprotein assays (LMP technology) can detect multiple phosphoprotein targets from a single cell lysate sample, they are a useful tool to reveal the phosphorylation state of targeted proteins along their signal transduction pathways. We used Bio-Plex phosphoprotein assay to probe the phosphorylation state of three NSCLC cell lines treated by gefitinib followed by single cell lysate sample. They are a useful tool to reveal the phosphorylation state of targeted reporters. Among three NSCLC cell lines, the wild type (H-1734) showed modest inhibition of phosphorylation on the downstream targets by gefitinib but its effect varies among three cell cultures (H-1734, H-1975, H-1650). Gefitinib inhibits the EGFR pathway but its effect varies among three cell cultures (H-1734, H-1975, H-1650).

Experimental Design

We tested the effects of gefitinib on six downstream targets of EGFR in three NSCLC cell lines with and without gefitinib treatment. For all tested cell lysates, the MFI of the Bio-Plex assay correlated very well with the band intensity on western blot. The multiplex assay needed only 10 µg of cell lysate protein for each sample. Western blot analysis needed 60 µg of protein per sample because each target was probed with individual anti-phospho-specific antibody.

Results

The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples.

Table 1. Magnitude of gefitinib inhibitory effect on six phosphoprotein targets in three NSCLC cell lines. Numbers represent the fold decline in phosphorylation measured between EGF and EGF + gefitinib lysates, calculated using MFI from the Bio-Plex phosphoprotein assay

<table>
<thead>
<tr>
<th>Cell Line</th>
<th>p-Akt</th>
<th>p-MEK1</th>
<th>p-ERK1/2</th>
<th>p-GSK-3</th>
<th>p-p70 S6K</th>
<th>p-p90RSK</th>
</tr>
</thead>
<tbody>
<tr>
<td>H-1734</td>
<td>15.0</td>
<td>45.0</td>
<td>20.4</td>
<td>4.7</td>
<td>11.5</td>
<td>18.9</td>
</tr>
<tr>
<td>H-1975</td>
<td>8.0</td>
<td>4.3</td>
<td>5.6</td>
<td>6.3</td>
<td>3.3</td>
<td>4.0</td>
</tr>
<tr>
<td>H-1650</td>
<td>1.4</td>
<td>0.9</td>
<td>0.8</td>
<td>1.0</td>
<td>1.0</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Among three NSCLC cell lines, the wild type (H-1734) showed moderate inhibition of phosphorylation on the downstream targets by gefitinib. The cell line H-1975, mutated by a deletion from L747 to P753, showed dramatic phosphorylation inhibition on all tested targets. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples from 4-fold to 45-fold (Table 1). However, cell line H-1650, which carries the double point mutation (L858R and T790M), did not respond to the drug with the exception of p-Akt, which showed a slight signal decrease between EGF- and gefitinib/EGF-treated samples (1.4-fold). No other targets showed inhibition by gefitinib. These results correspond with studies showing that the second point mutation (T790M) reverses the drug response because of the substitution of methionine for threonine at position 790, resulting in steric hindrance of the drug binding to EGFR.

Gefitinib phosphoprotein assays are useful tools in studying signal transduction pathways and measuring the phosphorylation status of multiple targets in anticancer drug discovery.

References


From https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2603198/

Fig. 1. Schematic of the signal transduction pathway downstream of EGFR. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples. The Bio-Plex assay measured signal decreases between EGF- and gefitinib/EGF-treated samples.

Fig. 2. Experimental Design

Fig. 3. Downstream EGFR Protein Phosphorylation and Gefitinib Inhibition in Non-Small Cell Lung Cancer Cells Detected With Multiplex Phosphoprotein Assays

Fig. 4. Results of the multiple phosphoprotein assays to drug discovery based on signal transduction pathways. Gefitinib, an inhibitor of epidermal growth factor receptor (EGFR), is used to treat non-small cell lung cancer (NSCLC). The responses of NSCLC to gefitinib are related to mutations occurring within the EGFR kinase domain. Because Bio-Plex phosphoprotein assays (LMP technology) can detect multiple phosphoprotein targets from a single cell lysate sample, they are a useful tool to reveal the phosphorylation state of targeted proteins along their signal transduction pathways. We used Bio-Plex phosphoprotein assay to probe the phosphorylation state of three NSCLC cell lines treated by gefitinib followed by single cell lysate sample. They are a useful tool to reveal the phosphorylation state of targeted reporters. Among three NSCLC cell lines, the wild type (H-1734) showed modest inhibition of phosphorylation on the downstream targets by gefitinib but its effect varies among three cell cultures (H-1734, H-1975, H-1650).

Fig. 5. Magnitude of gefitinib inhibitory effect on six phosphoprotein targets in three NSCLC cell lines. Numbers represent the fold decline in phosphorylation measured between EGF and EGF + gefitinib lysates, calculated using MFI from the Bio-Plex phosphoprotein assay.