Identification of Novel Chemical Modulators of the Estrogen Receptor α (ERα) in a MCF-7 Gene Expression Compendium

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Abstract
Identification of chemicals that affect hormone-regulated systems will help to predict endocrine disruption (ED). In our previous study (Ryan et al. 2016), ToxC1 151(1)(88-103), a gene expression biomarker was found to be an accurate predictor of estrogen receptor (ER) α modulation in chemically treated MCF-7 cells. Here, potential ERα modulators were identified by using the biomarker to screen a microarray compendium consisting of ~2300 gene expression profiles representing exposure to 1211 chemicals including those in the Connectivity Map (CMAP) 2.0 collection. A total of 86 and 54 chemicals were predicted to activate or suppress ERα, and they included many known ERα agonists and antagonists, respectively. The majority of the identified chemicals were also detected as active in an ERα trans-activation assay carried out in the human estrogen receptor cell line (BG1) used to screen the ToxC1 10.5K chemical library in agonist or antagonist modes. Chemicals predicted to modulate ERα in MCF-7 but not BG1 cells were examined further using trans-activation assays in MCF-7 cells, as well as cell free assays which measure interactions between ERα and co-regulators. These validation studies uncovered the following novel findings: 1) two chemicals were found to activate ERα but were inactive in the interaction assays, 2) progesterone receptor (PR) agonist activated ERα while the one PR antagonist (FLX0416) suppressed ERα, and 3) agonists for the liver X receptor and glucocorticoid receptor suppressed ERα indirectly. Our novel microarray screening strategy identified novel chemical modulators of ERα, some of which do not appear to act like classical agonists uncovering further evidence of the diversity of mechanisms that can modulate ERα. This primer does not represent EPA policy.

Methods
Biomarker Testing and Screening

Biomarker

Identification

ERα changes are measured using the Biomarker Testing and Screening tool of the Connectivity Map (CMAP) dataset.

Results

Table 1: Balanced accuracy calculation. "True positive" and "true negative" chemical classifications are based on results of the Tox21 ERα luc BG1 HTS assay. Predicted ERα agonists were identified as either "true positive" or "false negative" and predicted antagonists were identified as "true negative" or "false positive" chemical classifications respectively. Predicted SARs were evaluated relative to their respective study. (α = 0.05; β = 0.10).

<table>
<thead>
<tr>
<th>ERα Modulator</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Balanced Accuracy</th>
</tr>
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<tbody>
<tr>
<td>A</td>
<td>57.6%</td>
<td>96.9%</td>
<td>48.7%</td>
<td>97.8%</td>
<td>77.2%</td>
</tr>
<tr>
<td>B</td>
<td>57.1%</td>
<td>97.8%</td>
<td>36.4%</td>
<td>99.1%</td>
<td>77.5%</td>
</tr>
</tbody>
</table>

Conclusions
In the present study, we showed a compendium of MCF-7 gene expression profiles in an effort to identify activators and suppressors of ERα. We found: All chemical activation and 94 suppression of ERα, many of which are novel ERα modulators.

- Activators included known ERα agonists, progesterone receptor agonists and other steroids.
- Over half of the chemicals identified in the Tox21 ERα luc HTS assay in BG1 cells were also identified in our screen.
- The comparisons between Tox21 and biomarker screens identified chemicals that were active in the Tox21 assays but not using the biomarker approach (potential false negative).
- The biomarker approach also identified chemicals that were negative in the HTS assay of which were selected for further investigation.
- Validation experiments suggested the potential for weak ERα activation by ~2 of the chemicals, yet none altered co-activator binding or ERα in agonist or antagonist modes.
- Experiments are currently underway to confirm the gene expression changes induced by these potential ERα activators.

Figure 4: Visualization of biomarker –Log (p) values vs. Tox21 HTS assay results. Indicated chemicals were selected for further investigation.

Figure 5: Identification of chemicals that activate ERα but do not alter interactions with co-regulator proteins.

Figure 2: Compounds not analyzed in the CMAP dataset which modulate ERα in MCF-7 cells.

Figure 3: Compounds in the CMAP dataset which modulate ERα in MCF-7 cells.