High-throughput screening of the hypoxia pathway: examining promoter response and function

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INTRODUCTION

Many studies have highlighted the benefit of conducting genome-wide expression and transcription factor binding studies in parallel. However, after generating these descriptive data sets, a number of questions remain. Which genomic elements are responsible for transcript level changes and what is the effect of a transcription factor binding event? Using functional reporter assays to study the behavior of genomic elements in living cells can help answer many of these questions. To enable these studies in a high-throughput format, we have created a genome-wide library of human promoters and UTRs in a luciferase-based reporter system. Our unique collection of validated assays allows you to measure gene expression changes associated with a number of disease-related biological pathways, including hypoxia.

The hypoxia pathway, mediated through the hypoxia-inducible factor 1 (HIF-1), is essential to normal growth and development and is involved in the pathophysiology of cancer, inflammation and ischemia. Hypoxic conditions in tumors also cause resistance to radiotherapy and chemotherapy, creating considerable interest in the development of anti-hypoxia drugs. Although HIF-1 inhibition in hypoxic tumor cells may confer therapeutic benefits, evaluating the full HIF-1 pathway is critical for efficient screening of small molecules and minimizing off-target effects. In this study, we assembled a panel of known and potentially novel hypoxia-related promoters to measure the response and determine the function of these promoters, including naturally occurring sequence variants, to different treatments and conditions in high-throughput.

The LightSwitch™ System for pathway studies

DISEASE PROFILING PANEL

Pathway activity readout for:

- Hypoxia
- NFkB
- Inflammation
- ER
- Estrogen
- NFkB
- Inflammation
- STAT
- Interferon
- Androgen
- Hypoxia
- p300
- MAPK
- TNF
- NFkB
- Serotonin receptor
- Estrogen
- AR
- Androgen
- TR
- TSS
- 1 kb fragment
- Dist.

Toxicology screen of the hypoxia pathway

Identification of Chemical Compounds that Induce HIF-1α Activity

<table>
<thead>
<tr>
<th>Compound</th>
<th>IC50 (μM)</th>
<th>HIF-1α Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Caffeine</td>
<td>5.2</td>
<td>28%</td>
</tr>
<tr>
<td>B. Naringenin</td>
<td>4.1</td>
<td>29%</td>
</tr>
<tr>
<td>C. DFO</td>
<td>3.9</td>
<td>30%</td>
</tr>
<tr>
<td>D. 6. 7-Diethylamino-4-methylcoumarin</td>
<td>2.8</td>
<td>26%</td>
</tr>
</tbody>
</table>

Figure 6. Screening a toxicology panel for HIF-1α-dependent hypoxia inducers. Table: A primary reporter screen identified 15 of 1408 compounds that activate a synthetic hypoxia response element (HRE). A secondary VEGF secretion assay, the LightSwitch hypoxia pathway screen confirms that compounds 6 and 7 are outliers as they do not induce HRE-containing promoter activity.

CONCLUSIONS

- The LightSwitch System demonstrates optimal performance when mapping and measuring the behavior of hypoxia-responsive genomic elements
- HIF-1α motif and sequence variant experiments identified regulatory elements necessary for hypoxia response
- A high-throughput hypoxia screen allowed new insight into the regulation of the human hypoxia pathway, including the identification of several HIF-1α-dependent hypoxia inducers from a toxicology panel