Induction of the inflammatory regulator A20 by gibberellic acid in airway epithelial cells


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DATA SUPPLEMENT

INDUCTION OF THE INFLAMMATORY REGULATOR A20 BY GIBBERELLIC ACID IN AIRWAY EPITHELIAL CELLS

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Key Words: A20 protein; NF-kappaB; Gibberelic Acid, Airway epithelial cells; Inflammation.
EXPERIMENTAL PROCEDURES

Quantitative real time qPCR

Primers were designed using gene accession numbers and Primer3 open-source PCR primer design software and obtained from Invitrogen Ltd. (Paisley, UK). Primer sequences are given in Table S1.

Table S1: PCR primer sequences.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Accession Number</th>
<th>Sequence</th>
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<tr>
<td>Beta-actin</td>
<td>NM_001101.3</td>
<td>5’ctcttccagcctctctctc 3’&lt;br&gt;3’agcactgtgtggtgctacg 5’</td>
</tr>
<tr>
<td>A20</td>
<td>NM_006290</td>
<td>5’gagagcacaatggtgaaca 3’&lt;br&gt;3’tccagtgtgtatcgggtcat 5’</td>
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<tr>
<td>p65</td>
<td>NM_021975</td>
<td>5’cctggagcaggctatcgctc 3’&lt;br&gt;3’cactgtcactgtggaacgca 5’</td>
</tr>
<tr>
<td>TLR4</td>
<td>NM_003266.3</td>
<td>5’tggacaatttttgctagagg 3’&lt;br&gt;3’gatccagcccatgtgctt 5’</td>
</tr>
</tbody>
</table>
SUPPLEMENTARY RESULTS

Cell Proliferation

**Figure S1: GA₃ does not induce cellular proliferation.** 16HBE14o- cells were incubated with GA₃ (0-300 µM) for 72h and cell proliferation was determined in using the CellTiter 96® AQueous One Solution Cell Proliferation Assay (Promega, UK) according to the manufacturer’s recommendations. No change in proliferation was observed.

Purity of GA₃ preparation - Expression of TLR4 mRNA

**Figure S2: GA₃ preparation does not induce TLR4 mRNA expression.** To determine if the GA₃ preparation used in this study may contain traces of endotoxin, expression of TLR4 and β-actin (housekeeping gene) were assessed by qPCR. LPS alone [10 µg/ml] significantly increased TLR4 mRNA expression in a time dependent manner (p<0.01 LPS 1h vs. LPS 24h, Kruskal Wallis with Dunn’s post hoc test, n=5), but exposure of cells to GA₃ [30 µM] did not induce TLR4.