Supplemental material

S6K1 negatively regulates TAK1 activity in the TLR signaling pathway

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Inventory of Supplemental material

Supplemental material provides 3 supplemental Tables, and supplemental Materials and methods

Supplementary Methods

Materials and Methods

Microarray analysis

For microarray analysis, Control THP-1 cells were treated with or without LPS (100 ng/ml) and FSL-1 (10 μg/ml) for 3 h or 9 h. Total RNA was extracted using Trizol (Invitrogen Life Technologies, Carlsbad, CA, USA) and purified using RNeasy columns (Qiagen, Valencia, CA, USA) according to the manufacturers' protocol. After processing with DNase digestion, and clean-up procedures, RNA samples were quantified, aliquoted, and stored at −80 °C until use. For quality control, RNA purity and integrity were evaluated by denaturing gel electrophoresis, OD 260/280 ratio, and analyzed on Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA, USA). Total RNA was amplified and purified using the Ambion Illumina RNA amplification kit (Ambion, Austin, TX, USA) to yield biotinylated cRNA according to the manufacturer's instructions. Briefly, 550 ng of total RNA was reverse-transcribed to cDNA using a T7 oligo (dT) primer. Second-strand cDNA was synthesized, *in vitro* transcribed, and labeled with biotin-NTP. After purification, the cRNA was quantified using the ND-1000 Spectrophotometer (NanoDrop, Wilmington, NC, USA). Typically, 750 ng of labeled cRNA samples were hybridized to each humanHT-12 expression v.4 bead array for 16–18 h at 58 °C, according to the manufacturer's instructions (Illumina Inc., San Diego, CA, USA). Detection of array signal was carried out using Amersham fluorolink streptavidin-Cy3 (GE Healthcare Bio-Sciences, Little Chalfont, UK) following the bead array manual. Arrays were scanned with an Illumina bead array Reader confocal scanner according to the manufacturer's instructions. For raw data preparation and statistical analysis, the quality of hybridization and overall chip performance were monitored by visual inspection of both internal quality control checks and the raw scanned data. Raw data were extracted using the software provided by the manufacturer (Illumina GenomeStudio v2009.2 (Gene Expression Module v1.5.4)). Array data were filtered by detection of *P*-value <0.05 (similar to signal to noise) in at least 50% samples (we applied a filtering criterion for data analysis; higher signal value was required to obtain a detection *P*-value <0.05). Selected gene signal value was transformed by logarithm and normalized by quantile method. The comparative analysis between test sample and control sample was carried out using fold change. Hierarchical

cluster analysis was performed using complete linkage and Euclidean distance as a measure of similarity. GO-ontology analysis for significant probe list was performed using PANTHER (http://www.pantherdb.org/panther/ontologies.jsp), using text files containing Gene ID list and accession number of illumina probe ID. Gene Set Enrichment Analysis was performed to check whether the *a priori* defined set of genes shows a differential pattern in both the biological process and the molecular function states. One-tail Fisher Exact was adopted to measure the gene enrichment in annotation terms. All the data analysis and visualization of differentially expressed genes was conducted using R 2.4.1(www.r-project.org). For analysis of NF-κB-dependent upregulated and downregulated genes, genes that contain κB-binding site were sorted (Table S1) and analyzed under different combinations; 1, Control THP-1 cells treated with LPS or FSL-1 for 3h *versus* Control THP-1 cells; 2, Control THP-1 cells treated with LPS or FSL-1 for 9h *versus* Control THP-1 cells; 3, $S6K1^{KD}$ THP-1 cells treated with LPS or FSL-1 for 3h *versus* $S6K1^{KD}$ THP-1 cells; 4, $S6K1^{KD}$ THP-1 cells treated with LPS or FSL-1 for 9h *versus* $S6K1^{KD}$ THP-1 cells; 5, $S6K1^{KD}$ THP-1 cells *versus* Control THP-1 cells; 6, $S6K1^{KD}$ THP-1 cells treated with LPS or FSL-1 for 3h *versus* Control THP-1 cells treated with LPS or FSL-1 for 3h; 7, $S6K1^{KD}$ THP-1 cells treated with LPS or FSL-1 for 9h *versus* Control THP-1 cells treated with LPS or FSL-1 for 9h cells.

Supplementary Tables

Supplemental Table S1: The summary of microarray results of up- or down-regulated NF-kB genes induced by TLR2 or TLR4 stimulation in control and $S6K1^{KD}$ THP-1 cells
; *The result was provided by Excel file as Supplemental Table S1*

Supplemental Table S2: The NF-κB-dependent up-regulated and down-regulated genes induced by TLR4 stimulation in control or $S6K1^{KD}$ THP-1 cells.

Supplemental Table S3: The NF-κB-dependent up-regulated and down-regulated genes induced by TLR2 stimulation in control or $S6K1^{KD}$ THP-1 cells