SUPPLEMENTARY MATERIALS

Measurement of DNA synthesis by bromodeoxyuridine incorporation enzyme-linked immunosorbent assay

Cellular DNA synthesis was determined by estimating the amount of bromodeoxyuridine (BrdU) incorporated into DNA using a colorimetric immunoassay (Roche Diagnostics GmbH, Mannheim, Germany). Briefly, cells were plated at a density of 1×10^4 cells/ml in 96-well plates. Subconfluent cell cultures were treated with different concentrations of simvastatin and NS-398 for 24 hours. During the last 18 hours of the experiment, the cells were labeled with 10 μM BrdU and incorporation was measured according to the manufacturer's instructions (Roche Diagnostics). Results are expressed as percent inhibition of BrdU incorporation compared to the control. Cell growth was determined by trypan blue staining. After 24 hours of serum starvation, the cells were treated with simvastatin and NS-398 for 24 hours and stained with trypan blue. Viable cells were counted in three different 6,200× power fields, and the percentage of viable cells of the total number of counted cells was determined.

Liver function tests

The blood of each mouse was collected, and the serum was separated. The activities of alanine aminotransferase and aspartate aminotransferase were analyzed at the Green Cross Reference Laboratory (Yongin, Korea).

Histopathological examination

Liver tissue was fixed in 10% neutral-buffered formalin solution for no more than 24 hours, dehydrated in a graded alcohol series, cleared in xylene, and embedded in paraffin. The histopathological examination was performed using hematoxylin and eosin and Masson's trichrome stains. For the quantitative analysis of fibrosis in the liver, Masson's trichrome positive areas were measured using Image J 1.44 image analysis software (National Institute of Health, Bethesda, MD, USA) and a CX41 microscope (Olympus, Tokyo, Japan) with a DP21 digital camera (Olympus). The amount of collagen deposited was calculated from 25 randomized and non-overlapping areas at 920× magnification.

Quantification of collagen contents by hydroxyproline assay

Total collagen was determined by quantifying hydroxyproline content following the manufacturer's instructions (BioVision Inc., Milpitas, CA, USA). Mouse liver tissue was hydrolyzed with 6 N HCl at 110°C for 14 hours. The hydrolysates were filtered through 45-mm pore filters (Millipore, Darmstadt, Germany) and dried under a vacuum. The samples and hydroxyproline standards were incubated with chloramine-T buffer for 10 minutes at room temperature. Ehrlich's reagent was added, and the samples were incubated again for 45 minutes at 65°C. Absorbance of each sample was measured at 450 nm using a microplate reader (Packard BioScience, Meriden, CT, USA). Hydroxyproline levels were calculated against a 4-hydroxy-L-proline standard curve (Biovision) and expressed as mg hydroxyproline per gram liver tissue.

RNA extraction and real-time polymerase chain reaction

Total RNA was isolated from frozen liver tissue with the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. First-strand cDNA was generated with reverse transcriptase (Promega, Madison, WI, USA) using random primers (Promega). The polymerase chain reaction products were amplified with rTaq (Roche). Expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used as an internal control. Specific primers for tissue inhibitor of matrix metalloproteinases (TIMP)-1, TIMP-2, matrix metalloproteinase (MMP)-1, and MMP-2 were designed from their GenBank sequences and synthesized by BioBasic Inc. (Markham, ON, Canada) (Supplementary Table 2).