and 24 h. Cell numbers were measured by the trypan blue exclusion method. Cell pellets were stored at -80 °C until further PLTP expression assay.

SDS-PAGE and Western Blotting

The total cellular protein contents in the conditioned medium and HepG2 cells were analyzed by BCA assay.[13] Proteins in the conditioned medium and cell suspension were then separated by 10% SDS-PAGE. The conditioned medium (50 μg protein) or cell suspension (200 μg protein) was mixed with an equal volume of 2X SDS-PAGE sample buffer (0.125 M Tris-HCl, pH 6.8, 4% SDS, 20% glycerol, 10% 2-mercaptoethanol),[14] denatured at 100 °C for 3 min, and subjected to 10% SDS-PAGE (140 × 160 × 1.5 mm, SE 400, Hoefer Pharmacia Biotech, Taipei, Taiwan) with 4% stacking gel. The gel was run in tank buffer (25 mM Tris, pH 8.3, 192 mM glycine, 0.1% SDS) at 40 V for 18 to 19 h. Proteins in the conditioned medium after 10% SDS-PAGE were visualized by Coomassie blue staining. Proteins in the cell suspension were then transferred onto a nitrocellulose membrane in Towbin buffer (25 mM Tris, 192 mM glycine, 1.3 mM SDS, 10% methanol)[15] at 200 mA for 2 h using a semi-dry transfer unit (TE 70, Hoefer Pharmacia Biotech). The bands were visualized by 0.2% Ponceus S (Acros Organics, Taipei, Taiwan)/3% trichloroacetic acid (Lancaster Synthesis, Taipei, Taiwan) staining to mark the positions of the protein standards. The membrane was then washed briefly with PBS, and incubated with blocking buffer (0.2% casein, 0.1% Tween-20 in PBS) for 1 h. After blocking, the membrane was incubated with 6 μg/mL mouse anti-human monoclonal PLTP antibody (generously provided by Dr. An-Yue Tu, Univ. of Washington, Seattle, WA) at room temperature overnight. The membrane was washed twice with 0.1% Tween-20 in PBS, and incubated with goat anti-mouse IgG-alkaline phosphatase conjugate (Tropix) at 1:5000 dilution for 1.5 h. The blot was washed 3 times with 0.1% Tween-20 in PBS followed by twice with assay buffer (20 mM Tris-HCl, pH 9.8, 1 mM $MgCl_2$). Finally, the blot was incubated with CDP-Star™ (dioxetane) (Tropix) for 10 min and exposed to x-ray film. The chemiluminescent signals were analyzed by an image analysis system (Gel analysis system, EverGene Biotechnology, Taipei, Taiwan) and Phoretix 1D Lite gel analysis software (Phoretix International, Newcastle, UK).

PLTP mRNA Expression

Total cellular RNA was extracted by salt precipitation using a PUREscript RNA isolation kit.[16,17] The tube containing 5×10^6 cells was placed on ice. The cell pellet was resuspended by adding 900 mL cell lysis solution. The cell lysate was mixed with 300 mL of protein-DNA precipitation solution on ice for 10 min, and centrifuged at 15,000 xg (Zentrifugen EBA 12R, Hettich, Tuttlingen, Germany) for 5 min at 4 °C. The supernatant was mixed well with 750 μL of cold 100% isopropanol and placed at -20 °C for 10 min. After being centrifuged at 15,000 xg for 5 min at 4 °C, the pellet was washed with 750 μL of cold 70% ethanol, and allowed to air dry for 10 -15 min. Finally, the RNA pellet was rehydrated on ice in 15 μL of RNA hydration solution. The concentration (μg/10^7 cells) and purity of total cellular RNA were determined spectrophotometrically (U-2000 spectrophotometer, Hitachi, Tokyo, Japan) by absorbance at 260 nm and the ratio of absorbances at 260 nm and 280 nm, respectively.

PLTP mRNA was measured by slot blotting. Total cellular RNA (30 μg) was mixed with sample loading buffer (1X MOPS buffer, 50% formamide, 2.0 M formaldehyde, 1 μg ethidium bromide, 0.02% bromophenol blue, 5% glycerol). Denatured RNA was directly applied on Duralon-UV nylon membranes (Stratagene) using a Hoefer PR648 slot blot filtration apparatus. After being UV cross-linked (Ultra-Lum UVC-508 UV crosslinker, Ultra-Lum, Carson, CA) and air dried, PLTP mRNA on the membrane was hybridized with a human fluorescein-labeled PLTP cDNA probe (1294 bp), purified from pZem 228cc plasmid (generously provided by Dr. An-Yue Tu, Univ. of Washington, Seattle, WA) after Sst I restriction enzyme digestion, in the hybridization buffer (1% BSA, 7% SDS, 0.25 M NaCl, 1.0 mM EDTA, 0.45 M Na_2HPO_4, pH 7.2) at 68 °C overnight. The membrane was washed several times with 0.1X SSC and 1% SDS solution followed by TBSN solution (50 mM Tris-HCl, pH 8.0, 150 mM NaCl, 0.01% Northern block reagent, Illuminator™ chemiluminescent detection system, Stratagene) to remove SDS. Prior to incubation with antifluorescein antibody-alkaline phosphatase conjugate at 1:10000 dilution at room temperature for 1 h, the DNA-RNA hybrid was incubated with blocking buffer (0.2% Northern