Expression Cloning and Characterization of a Novel Glycosylphosphatidylinositol-anchored High Density Lipoprotein-binding Protein, GPI-HBP1*

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By expression cloning using fluorescent-labeled high density lipoprotein (HDL), we isolated two clones that conferred the cell surface binding of HDL. Nucleotide sequence of the two clones revealed that one corresponds to scavenger receptor class B, type 1 (SRBI) and the other encoded a novel protein with 228 amino acids. The primary structure of the newly identified HDL-binding protein resembles GPI-anchored proteins consisting of an N-terminal signal sequence, an acidic region with a cluster of aspartate and glutamate residues, an Ly-6 motif highly conserved among the lymphocyte antigen family, and a C-terminal hydrophobic region. This newly identified HDL-binding protein designated GPI-anchored HDL-binding protein 1 (GPI-HBP1), was susceptible to phosphatidylinositol-specific phospholipase C treatment and binds HDL with high affinity (calculated $K_d = 2–3 \mu M$). Similar to SRBI, GPI-HBP1 mediates selective lipid uptake but not the protein component of HDL. Among various ligands for SRBI, HDL was most preferentially bound to GPI-HBP1. In contrast to SRBI, GPI-HBP1 lacked HDL-dependent cholesterol efflux. The GPI-HBP1 transcripts were detected with the highest levels in heart and, to a much lesser extent, in lung and liver. In situ hybridization revealed the accumulation of GPI-HBP1 transcripts in cardiac muscle cells, hepatic Kupffer cells and sinusoidal endothelium, and bronchial epithelium and alveolar macrophages in the lung.

High density lipoprotein (HDL) plays a key role in the transportation of cholesterol to extrahepatic tissues including

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The abbreviations used are: HDL, high density lipoprotein; LDL, low density lipoprotein; BSA, bovine serum albumin; CHO, Chinese steroidogenic tissues and in the reverse transportation of cholesterol from extrahepatic tissues to the liver (1). Unlike the low density lipoprotein (LDL) receptor pathway, the delivery of cholesterol from HDL to cells is mediated by selective lipid uptake from HDL particles and is independent of internalization of HDL. Reverse cholesterol transportation requires the extraction of cholesterol from extrahepatic cells by HDL and the subsequent delivery of cholesterol to hepatocytes.

Several HDL-binding proteins have been identified including class B type I scavenger receptor (SRBI) (2, 3), two candidate hepatic HDL receptors designated HDL-binding proteins 1 and 2 (4, 5), 80- and 130-kDa GPI-anchored HDL-binding proteins expressed in human macrophages (6), 110-kDa GPI-anchored HDL-binding protein expressed in HepG2 cells (7), and recently characterized 95-kDa HDL-binding protein (8). To date, only SRBI appears to be a physiological HDL receptor based on the selective uptake of cholesterol esters into cells and the efflux of cholesterol from cells to HDL mediated by SRBI (1). Consistent with the postulated physiological role, SRBI is highly expressed in tissues that selectively take up cholesterol esters from HDL including liver, adrenal gland, testis, and ovary (3). Although hepatic overexpression of SRBI mediated by an adenovirus encoding SRBI resulted in a dramatic reduction of plasma cholesterol (9), the targeted disruption of the murine SRBI gene led to a modest increase in plasma cholesterol (10). This finding may suggest the presence of other HDL receptors that cooperate with SRBI in the metabolism of HDL.

In this study, we identified a novel HDL-binding protein by expression cloning from a murine hepatic cDNA library. This newly identified HDL-binding protein designated GPI-anchored HDL-binding protein 1 (GPI-HBP1) belongs to the GPI-anchored lymphocyte differentiation antigen Ly-6 family, binds HDL with high affinity on the cell surface, and mediates selective lipid uptake from HDL particles and is independent of internalization from HDL particles. We also describe the ligand specificity and tissue expression of GPI-HBP1.

hamster ovary; Dil, 1,1′-dioctadecyl-3,3,3′,3′-tetramethylindocarbocyanine perchlorate; GPI, glycosylphosphatidylinositol; HBPl, HDL-binding protein 1; Ly-6, lymphocyte antigen 6; PBS, phosphate-buffered saline; PIPLC, phosphatidylinositol-specific phospholipase C; SRBI, scavenger receptor class B, type I; sHBP1, soluble HDL-binding protein 1.
**EXPERIMENTAL PROCEDURES**

**Materials**—Human HDL, LDL, acetylated LDL and oxidized LDL, and newborn calf lipoprotein-deficient sera were prepared essentially as described previously (2, 11). GPI-HBP1 cDNA was prepared according to the procedure as described previously (12). Oxidized LDL was prepared by dialyzing 1 ml of LDL (4–10 mg/ml) (prepared without β-hydroxybutyrate) against saline solution containing 5 μM CuSO₄ (2 × 500 ml) for 24–48 h at 4 °C. 1,1-Dioctadecyl-3,3,3,3-tetramethylindocarbocyanine perchlorate (DiI) was obtained from Molecular Probes. Bacillus cereus phospholipase C (Bacillus cereus, specific activity of 7.1 units/mg), egg yolk phosphatidylcholine, and brain phosphatidylserine were from Sigma. Phospholipid liposomes were prepared by extrusion through polycarbonate membranes as described previously (14): phosphatidylcholine liposomes (phosphatidylcholine/free-cholesterol, molar ratio 2:1) and phosphatidylserine liposomes (phosphatidylcholine/phosphatidylserine/free-cholesterol, molar ratio 1:1:1).

**Standard Procedures**—Standard molecular biology techniques were performed essentially as described by Sambrook and Russell (15). CDNA clones were subcloned into pBluescript vectors and sequenced by the dideoxy chain-termination method with a BigDye Terminator Cycle Sequencing Ready Reaction kit (PE Biosystems) and a DNA sequencer (model 310, PE Biosystems). To analyze RNA in murine tissues, commercially available Northern blots (Clontech Laboratories) were used for Northern blot analysis. ³²P-Labeled probes were prepared by priming with random hexanucleotides.

**Expression Cloning**—A cDNA library was constructed from poly(A) RNA isolated from the livers of LDL receptor-deficient male mice (16) in the pZeoSV2 vector (Invitrogen) using a NoI unidirectional primer. The cDNA library consisted with ~3 × 10⁷ clones, and these clones were divided into small pools (300 clones/pool). Plasmid DNA from each pool was prepared using the QIAprep 96 Turbo Miniprep kit (Qiagen). On day 0, LDL receptor-lacking Chinese ovary cells, ldlA7 (17), were plated into 96-well plates (5 × 10⁴ cells/well) in minimum essential medium supplemented with 5% fetal bovine serum and 250 μg/ml gentamycin. Positive pools were serially subdivided and restested to obtain positive cDNA clones.

**Cell Culture and Transfection**—The entire coding regions of murine GPI-HBP1 and SRBI were subcloned into the pRC/CMV vector (Invitrogen) for stable transfection. CHO ldlA7 cells were transfected with the expression plasmid using the Lipofectamine reagent (Invitrogen) according to the manufacturer's instructions. Stably transfected cells were selected in Ham's F-12 medium containing 50 units/ml bovine serum, 50 μg/ml streptomycin, and 2 mM glutamine (medium B) supplemented with 5% fetal bovine serum and 250 μg/ml G418 for 2 weeks. For III-1-HDL and II-HDL binding studies and [³¹]Cholesterol efflux assays (see below), cells were plated in 6-well (250,000 cells/well) dishes in medium B supplemented with 5% newborn calf lipoprotein-deficient serum and cultured for 48 h.

**Phosphatidylinositol-specific Phospholipase C (PIPLC) Treatment**—GPI-HBP1-expressing cells were incubated for 1 h in medium B supplemented with 5% newborn calf lipoprotein-deficient serum with or without PIPLC at 37 °C. After a 1-h incubation with or without PIPLC at 37 °C, cells were washed and incubated for the measurement of DiI-HDL uptake. The amount of cell-associated DiI was determined as described under "Experimental Procedures." Values are the average of triplicate determinations, and error bars represent the range of the three measurements.
GPI-anchored HDL-binding Protein

Fig. 4. Concentration dependence of HDL binding to and HDL lipid uptake by GPI-HBP1 and SRBI-expressing cells. CHO IdlA7 cells were stably transfected with expression vectors for murine GPI-HBP1, murine SRBI, or the control (“parental”) vector (pRC/CMV) as described under “Experimental Procedures.” Cells were plated in six-well dishes and incubated with the indicated amounts of either 125I-HDL (A and B) or DiI-HDL (C and D) at either 4°C (A and C) or 37°C for 2 h. The amounts of specific 125I-HDL binding or DiI uptake (spectrofluorimetry) were determined by subtracting the values obtained with parental vector (pRC/CMV) transfected cells from those obtained with a given expression plasmid as described under “Experimental Procedures.” Similar results have been observed in multiple independent experiments, and the data shown are representative. Error bars represent the range of variations in the triplicate determinations.

without 1 unit/ml PIPPLC. Cells were then washed and incubated for the measurement of DiI-HDL uptake.

125I-HDL-binding and Association Assays—Cells were washed once with medium B and then re-fed with medium B containing 0.5% (w/v) fatty acid-free BSA and the indicated concentrations of 125I-HDL. After a 2-h incubation at 37°C, cells were washed once with 50 mM Tris-HCl, pH 7.4, and 0.15 M NaCl (buffer A) containing 2 mg/ml BSA followed by two quick washes with buffer A without BSA. Cells were then solubilized with 0.1 M NaOH for 30 min at room temperature on a shaker, and we then determined the amounts of cell-associated radioactivity using a γ-counter. The protein content was determined using the method of Lowry et al. (18). For 4°C binding studies, the protocol was identical to that at 37°C with the exception that the cells were prechilled on ice for 15 min and incubated with 125I-HDL at 4°C for 2 h. Specific cell association or binding was determined by subtracting the values obtained with parental vector (pRC/CMV) transfected cells from those obtained with a given expression plasmid.

Fluorimetric Assay of DiI-HDL Uptake—DiI-HDL was used to measure the cellular association and uptake of fluorescence by cells stably expressing GPI-HBP1 or SRBI according to the procedure as described by Acton et al. (3). Cells were washed once with medium B and then re-fed with medium B containing 0.5% (w/v) fatty acid-free BSA and the indicated concentrations of DiI-HDL. After incubation at 37°C for 2 h, cells were washed twice with PBS containing 5 mM CaCl2 and 5 mM MgCl2 (5 min/wash). Cell-associated DiI was then solubilized in 0.5 ml of Me2SO at room temperature for 2 h, and the fluorescence was measured using a spectrofluorometer. The amount of DiI in each sample expressed as equivalent amounts of DiI-HDL (micrograms of protein) was calculated by comparing the fluorescence intensity of the sample to that from a standard curve generated by dissolving DiI-HDL in Me2SO.

Fig. 5. Inhibition of 125I-HDL binding to GPI-HBP1 expressing cells by various compounds. GPI-HBP1 or SRBI expressing cells were incubated with 10 µg/ml 125I-HDL at 4°C for 2 h in the presence of 200 µg/ml of the indicated compound. PC, phosphatidylcholine; PS, phosphatidylserine; Ox-LDL, oxidized LDL; Ac-LDL, acetylated LDL. The 100% control values for the binding of 125I-HDL (in the absence of inhibitors) of GPI-HBP1- and SRBI-expressing cells were 48.9 and 190 ng/mg protein, respectively. Error bars represent the range of variations in the triplicate determinations.

Specific DiI uptake was determined by subtracting the values obtained with parental vector transfected cells from those obtained with a given expression plasmid.

13H-Cholesterol Efflux from Stably Transfected CHO IdlA7 Cells—HDL-dependent cholesterol efflux study was performed according to the procedure described by Gu et al. (19). Cells (~70% confluent) were incubated for 48 h with 0.2 µCi/ml [1,2-3H]cholesterol (40–60 Ci/mmol, Amersham Biosciences). After washing five times with PBS containing 1% fatty acid-free BSA, radiolabeled cells were incubated overnight in Ham’s F12 containing 1% fatty acid-free BSA to allow for the equilibration of cellular cholesterol pools. Cells were then washed and incubated for the indicated times in efflux medium (Ham’s F12, 0.5% fatty acid-free BSA) with or without 40 µg/ml HDL. The efflux medium was collected and clarified by centrifugation for 1 min with a Microcentrifuge, and the radioactivity of each supernatant was determined by liquid scintillation counting. Cells were solubilized with 1% Triton X-100 in PBS for 30 min at room temperature, and the amount of [3H]cholesterol in each lysate was determined. Total cellular [3H]cholesterol was calculated as the sum of the radioactivity in the efflux medium plus the radioactivity in the cells and was used to calculate the [3H]cholesterol efflux (percent of total [3H]cholesterol released into the medium).

In Situ Hybridization—Digoxigenin-11-UTP-labeled single-stranded RNA probes were prepared with a digoxin/RNA labeling mixture and the corresponding T3 or T7 RNA polymerase (Roche Molecular Biochemicals) according to the manufacturer’s instructions. The entire coding region of murine GPI-HBP1 cDNA was subcloned into the pBluescript II vector (Stratagene) and used to prepare single-stranded RNA probes. Tissues from a C57BL/6J male mouse (10–12 weeks old) were fixed in PBS containing 4% paraformaldehyde at 4°C for 12 h, dehydrated, and embedded in paraffin using a standard procedure. In situ hybridization was performed using 6-µm thick sections mounted on silane-coated glass slides. After digestion with 10 mg/ml proteinase K at 37°C for 20 min, the tissue sections were hybridized with antisense or sense RNA probes at 50°C for 16 h. For the reaction with antidigoxigenin antibodies, slides were washed with 100 mM Tris-HCl, pH 7.5 and 150 mM NaCl (buffer B), treated with 0.5% blocking reagent (Roche Molecular Biochemicals) in buffer B, and then incubated with alkaline phosphatase-coupled antidigoxigenin antibodies (diluted 1:750 in buffer B, Roche Molecular Biochemicals) for 1 h.
Expression Cloning of HDL-binding Proteins—We screened a cDNA library from murine liver for cDNAs that facilitate the binding of HDL when transiently expressed in LDL receptor-lacking ldlA7 cells. After screening a total of 960 pools of ~300 cDNA each, we obtained two pools of cDNAs that stimulated HDL binding to levels that were significantly higher than background. We then plated a total of 960 colonies from two positive pools into individual wells of 96-well plates. We prepared cDNAs from the pooled rows and columns of the plates. Four of these pools gave positive results. We then assayed individual clones from the wells at the intersections of the positive rows and columns and obtained two positive clones (Fig. 1). Nucleotide sequencing of these clones and subsequent BLAST search revealed that one corresponds to murine SRBI cDNA (3) and the other (designated pHRC7) encoded a protein of 228 amino acids (Fig. 2).

Structure of a GPI-anchored HDL-binding Protein—A hydrophathy plot of the deduced amino acid sequence of the cDNA shows the presence of two hydrophobic regions (Fig. 2), one at the N terminus and the other at the C terminus. The former corresponds to a classical signal sequence of probable 19 amino acids (Fig. 3), whereas the latter strongly resembles the hydrophobic region of GPI-anchored cell surface proteins. Excluding the N-terminal 19 amino acids, the mature protein would consist of 209 amino acids in length, whereas the latter strongly resembles the hydrophobic region of GPI-anchored cell surface proteins. Excluding the N-terminal 19 amino acids, the mature protein would then consist of 209 amino acids with a calculated Mr of 22,603. This value is greatly smaller than those values of the previously characterized candidate receptors for HDL (4–8).

The predicted amino acid sequence revealed the presence of a region highly enriched with acidic amino acids (aspartate and glutamate) and a region similar to a highly conserved domain termed Ly-6 motif, which occurs singly in the GPI-anchored lymphoid differentiation antigen Ly-6 family, and is repeated 3-fold in urokinase-type plasminogen activator receptor. These results predicted that the cloned protein consists of an N-terminal signal sequence, an acidic region with a cluster of aspartate and glutamate residues, an Ly-6 motif, and a C-terminal hydrophobic region.

Based on the structural similarity with the GPI-anchored Ly-6 family proteins, we analyzed the effects of phosphatidylinositol-specific PIPLC treatment on the HDL binding. As shown in Fig. 5, the PIPLC treatment almost completely abol-

Fig. 8. In situ hybridization analysis of GPI-HBP1 transcripts in mouse. Sections A, C, and E were hybridized with an antisense probe to murine GPI-HBP1. Sections B, D, and F are negative controls with a sense probe. Hybridization signals were visualized in blue. Tissue sections prepared from liver (A and B), heart (C and D), and lung (E and F) of a normal male mouse were analyzed by in situ hybridization as described under “Experimental Procedures.” GPI-HBP1 transcripts are localized in the Kupffer cells (indicated by arrows in A) and sinusoidal endothelium (arrowheads in A), but no significant accumulation is detected in the parenchymal cells. In the heart, the hybridization signal is detected in cardiac muscle cells (C). The intense hybridization signals are seen in bronchial epithelium (arrow in E) and alveolar macrophages (arrowhead in E) in the lung. Bars, 50 μm.
ished the uptake of DiI-HDL, suggesting that the cloned protein is indeed GPI-anchored. Therefore, we designate the cloned HDL-binding protein as GPI-HBP1.

EST data base search identified an isoform lacking the C-terminal half of GPI-HBP1. This isoform (designated sHBP1) shares the N-terminal signal sequence, the acidic region, and part of the Ly-6 motif with GPI-HBP1 but lacks the C-terminal hydrophobic region, suggesting that the isoform is a secreted form generated by alternative splicing. Consistent with the deduced structural feature, ldlA7 cells transiently transfected with sHBP1 failed to bind DiI-HDL on the cell surface (Fig. 1C).

BLAST searches of the GenBank™ databases revealed that GPI-HBP1 is structurally related to Ly-6 family proteins belonging within a superfamily that includes the urokinase-type plasminogen activator receptor, the complement inhibitor CD59, the sperm antigen SP10, and more distantly, the snake venom neurotoxin family. The Ly-6 motif consists of ~90 amino acids with ten highly conserved cysteine residues (20). A comparison of the sequence of the Ly-6 motif in GPI-HBP1 with those in the Ly-6 family members revealed that these cysteine residues are completely conserved in the Ly-6 motif of GPI-HBP1 (data not shown). The phylogenetic tree of the Ly-6 family proteins of various origins indicates that GPI-HBP1 is most closely related to Ly-6 molecules (data not shown).

HDL Binding—To characterize lipoprotein-binding properties, cDNAs encoding GPI-HBP1 and SRBI were stably expressed in ldlA7 cells. The binding of $^{125}\text{T}$ (DiI-HDL) or fluorescent (DiI-HDL) labeled HDL was measured following incubation of the cells. As shown in Fig. 4, A and B, the saturation binding of $^{125}\text{T}$-HDL was observed in both GPI-HBP1- and SRBI-expressing cells at 4 and 37 °C. Although the maximal binding of $^{125}\text{T}$-HDL in GPI-HBP1-expressing cells was 3-fold lower than that of SRBI-expressing cells, the calculated $K_d$ values of the two proteins were within the same range (2–3 μg/ml). The relative maximal binding activity between SRBI and GPI-HBP1 could not be determined because the expression levels of these two proteins in ldlA7 cells were unknown. Similar saturation binding was observed when cells were incubated with DiI-HDL at 4 °C (Fig. 4C). In contrast, when GPI-HBP1-expressing cells were incubated with DiI-HDL at 37 °C, the amounts of DiI-HDL binding by the cells were ~3-fold higher than those at 4 °C and unsaturable (Fig. 4D). Similarly, the amounts of DiI-HDL binding at 37 °C by SRBI-expressing cells were ~6-fold higher than those at 4 °C. Furthermore, compared with $^{125}\text{T}$-HDL binding, the degradation of $^{125}\text{T}$-HDL (trichloroacetic acid-soluble $^{125}\text{T}$) at 37 °C was almost negligible in both cells (data not shown). These data indicate that GPI-HBP1, similar to SRBI (3), mediates selective lipid uptake but not the protein component of HDL.

Effects of SRBI Ligands—We next analyzed the effects of various ligands for SRBI (2, 14) on the binding of $^{125}\text{T}$-HDL to GPI-HBP1-expressing cells. As shown in Fig. 5, in the presence of excess unlabeled HDL, the binding of $^{125}\text{T}$-HDL to GPI-HBP1- and SRBI-expressing cells was strongly reduced. Compared with the strong inhibition of $^{125}\text{T}$-HDL binding to SRBI-expressing cells by human apoAI (free form), phosphatidylserine, and acetylated LDL (inhibited by ~75%), the inhibitory effects by these compounds were relatively lower in GPI-HBP1-expressing cells (reduced by ~50%). Oxidized LDL, native LDL, and human apoAI had relatively weak inhibitory effects on $^{125}\text{T}$-HDL binding to GPI-HBP1- and SRBI-expressing cells. These data show that HDL is bound to GPI-HBP1 most preferentially among various SRBI ligands including HDL, phosphatidylserine, and acetylated LDL.

Cholesterol Efflux—In addition to selective lipid uptake activity, SRBI mediates HDL-dependent cholesterol efflux. To test whether GPI-HBP1 also mediates cholesterol efflux, the cells were labeled with $[^3\text{H}]$cholesterol, allowed for the equilibration of cellular cholesterol pools, and then incubated with or without HDL. As shown in Fig. 6, SRBI-expressing cells exhibited HDL-dependent cholesterol efflux with time-dependent manner, whereas almost no cholesterol efflux was seen in GPI-HBP1-expressing cells in the absence or the presence of HDL. The lack of cholesterol efflux in GPI-HBP1 cells indicates that GPI-HBP1 mediates selective lipid uptake only, whereas SRBI mediates both influx and efflux of cholesterol.

Expression of GPI-HBP1 Transcripts—Northern blot analysis of RNA from various murine tissues revealed hybridization of the GPI-HBP1 probe to a major transcript of 0.8 kilobases with the highest expression in heart and, to a much lesser extent, in lung and liver (Fig. 7). Apparently, no transcripts were detected in other tissues including brain, kidney, skeletal muscle, spleen, and testis.

To locate cells expressing GPI-HBP1 transcripts, in situ hybridization was performed using tissue sections from murine liver, heart, and lung. In the liver, the accumulation of hybridization signals for GPI-HBP1 transcripts appearing dark blue were detected most intensely in the Kupffer cells and sinusoidal endothelium, but no significant accumulation was detected in the parenchymal cells (Fig. 8, panel A). In the heart, the intense hybridization signal was detected in cardiac muscle cells (Fig. 8, panel C). The intense hybridization signals were also detected in bronchial epithelium and in alveolar macrophages in the lung (Fig. 8, panel E).

In contrast to the abundant expression of SRBI in steroidogenic tissues (3, 21), GPI-HBP1 transcripts were highly accumulated in the Kupffer cells as well as in alveolar macrophages of the lung. Based on the abundant expression in these scavenger cells and the lack of cholesterol efflux, it is suggested that GPI-HBP1 plays a role in the initial entry of HDL cholesterol into these scavenger cells for further transportation of cholesterol. To elucidate the precise biological role of GPI-HBP1 and to determine any disorders caused by the absence of the protein, the generation of mice lacking GPI-HBP1 is currently underway.

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REFERENCES

1. Krieger, M. (2001) J. Clin. Invest. 108, 793–797.
2. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., and Krieger, M. (1994) J. Biol. Chem. 269, 21003–21009.
3. Acton, S., Rigotti, A., Landschulz, K. T., Xu, S., Hobbs, H. H., and Krieger, M. (1996) Science 271, 518–520.
4. Tsuzuki, M., and Fidge, N. (1989) Biochem. J. 261, 239–244.
5. Matsuzato, A., Mitchell, A., Kurata, H., Pyle, L., Kondo, K., Itakura, H., and Fidge, N. (1997) J. Biol. Chem. 272, 16773–16782.
6. Matsuyama, A., Yamashita, S., Sakai, N., Maruyama, T., Okuda, E., Hirano, K., Ikihar, S., Hirakas, H., and Matsuwasu, Y. (2000) Biochem. Biophys. Res. Commun. 272, 864–871.
7. Nazih-Sanderson, F., Lestavel, S., Nion, S., Rouy, D., Denefle, P., Fruchart, J. C., Clavey, V., and Delbart, C. (1997) Biochem. Biophys. Acta 1358, 103–112.
8. Bocharov, A. V., Vishnyakova, T. G., Baranova, I. N., Patterson, A. P., and Eggerman, T. L. (2001) Biochemistry 40, 4407–4416.
9. Kozarsky, K. F., Donahue, M. H., Rigotti, A., Iqbal, S. N., Edelman, E. E., and Krieger, M. (1997) Nature 387, 414–417.
10. Rigotti, A., Triggati, B. L., Penman, M., Rayburn, H., Herz, J., and Krieger, M. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 12610–12615.
11. Goldstein, J. L., Basu, S. K., and Brown, M. S. (1983) Methods Enzymol. 98, 241–260.
12. Kim, D. H., Iijima, H., Goto, K., Sakai, R., Ishii, H., Kim, H. J., Suzuki, H., Kondo, H., Saeki, S., and Yamamoto, T. (1996) J. Biol. Chem. 271, 8373–8380.
13. Takahashi, S., Kawarabayasi, Y., Nakai, T., Sakai, J., and Yamamoto, T. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 9252–9256.
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14. Rigotti, A., Acton, S. L., and Krieger, M. (1995) *J. Biol. Chem.* **270**, 16221-16224

15. Sambrook, J., and Russell, D. W. (2001) in *Molecular Cloning: A Laboratory Manual*, 3rd Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY

16. Ishibashi, S., Brown, M. S., Goldstein, J. L., Gerard, R. D., Hammer, R. E., and Herz, J. (1993) *J. Clin. Invest.* **92**, 883-893

17. Kingsley, D. M., and Krieger, M. (1984) *Proc. Natl. Acad. Sci. U.S.A.* **81**, 5454-5458

18. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265-275

19. Gu, X., Kozarsky, K., and Krieger, M. (2000) *J. Biol. Chem.* **275**, 29993-30001

20. Shan, X., Bourdeau, A., Phoston, A., Wells, D. E., Calhoun, E. H., Landgraf, B. E., and Palfree, R. G. (1998) *J. Immunol.* **160**, 197-208

21. Rigotti, A., Edelman, E. R., Seifert, P., Iqbal, S. N., DeMattos, R. B., Temel, R. E., Krieger, M., and Williams, D. L. (1996) *J. Biol. Chem.* **271**, 33545-33549