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Rab7 gene is up-regulated by cholesterol-rich diet in the liver and artery☆

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Abstract

To identify genes responding to the cholesterol-rich diet, differentially expressed hepatic genes have been searched from a diet-induced hypercholesterolemic rabbit by differential display reverse transcription-polymerase chain reaction (DDRT-PCR). Among the many screened genes, Rab7 gene was shown to be distinctively up-regulated in response to the cholesterol-loading into the rabbit. To visualize the location of elevated Rab7 expression in tissues, patterns of the gene expression were monitored within hepatic and aortic tissues by in situ hybridization and immunohistochemistry. The expression of Rab7 was obviously increased in the hepatic tissues, especially in the endothelial cells and hepatocytes around central veins of the high cholesterol-fed rabbit, compared to the tissues from rabbit fed a normal diet. To find out a potential relationship between the Rab7 and the atherogenesis, the same experiments were conducted with the atherosclerotic plaques obtained from rabbit and human. The elevated expression of Rab7 gene was clearly evident in both tissues, suggesting that the Rab7 may be involved in the process of atherogenesis.

Keywords: Rab7; Atherosclerosis; Hypercholesterolemia; DDRT-PCR

It has been repeatedly shown that cholesterol directly modulates the transcription of many genes involved in the cholesterol metabolism and in the pathogenesis of atherosclerosis. For example, expression of hepatic apolipoprotein-B, -CII, -CIII, -E, cytochrome P450 isoenzyme, and Mac-2 was shown to be altered in cholesterol-fed rabbits [1–5]. However, more available information is needed on the regulation of gene expression in response to excess dietary cholesterol in the liver, because it is the major organ related to the lipoprotein synthesis, secretion and remodeling, and cholesterol clearance from circulation [6]. Hypercholesterolemia generated by a diet with high level of cholesterol causes fat deposition in the liver and depletion of hepatocyte population. It causes the malfunction of liver clearly, which is apparently presented through microvesicular steatosis due to the intracellular accumulation of lipids [7–10].

It has been demonstrated that there is a correlation between the hepatic cholesterol concentrations and the coronary heart diseases [11,12]. Davis and Hui [13] proposed that "atherosclerosis is a liver disease of the heart" because of the therapeutic importance of the liver in ameliorating hyperlipidemia and cardiovascular diseases. Accordingly, a change in the expression of key hepatic genes upon cholesterol-loading could be a

☆ *Abbreviations:* DDRT-PCR, differential display reverse transcription-polymerase chain reaction; GAPDH, glyceraldehydes-3-phosphate dehydrogenase; CRGRL, cholesterol-responding gene in rabbit liver; LDL, low-density lipoproteins; NBT, nitro blue tetrazolium chloride; BCIP, 5-bromo-4-chloro-3-indolyl phosphate; GDI, GDP-dissociation inhibitor.

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primary indication of the coronary heart diseases. To define appropriate genes specifically functioning at the sites of atherogenesis, attempts to search for those genes could be a valuable approach toward treatment and prevention of atherosclerosis and possibly toward decelerating the progress of the disease. To obtain more information related to those genes, cholesterol-responding hepatic genes were searched and characterized in diet-induced hypercholesterolemic rabbits using the differential display reverse transcription-polymerase chain reaction (DDRT-PCR) in this study. This technique allows comparison of expression levels of rare genes in a minute amount of mRNAs [14–16] and can be tested with a high probability for the detection of new differentially expressed cholesterol-responding genes, when it is compared to the previously described method of subtractive library construction [17,18].

One of the differentially expressed genes obtained in this study, Rab7, is up-regulated by the diet with a high level of cholesterol. Rab7, a ras-related Rab GTPase, is an important regulator in the proper aggregation and fusion of late-endocytic structures in the perinuclear region. Consequently, it plays a crucial role in the biogenesis and maintenance of lysosomal compartment [19,20]. Since low-density lipoproteins (LDL) follow the late-endosome/lysosome path when they are degraded, the Rab7 could be a key regulator in the process of LDL (or possibly oxidized LDL) breakdown. In this present study, we have shown an elevated expression of Rab7 in rabbit hepatic and aortic tissues in response to diet cholesterol. We have also observed the increased expression of Rab7 in the atherosclerotic plaque of human artery. This suggests that the Rab7 may be important in the pathological process of atherogenesis.

Materials and methods

Generation of hypercholesterolemic rabbits. Six male New Zealand white rabbits were fed ad libitum powdered chow diet supplemented with 2% (w/w) cholesterol for 12 weeks. Levels of the plasma cholesterol and triglyceride were measured every week by enzymatic colorimetric methods [21,22]. The rabbits on the cholesterol-supplemented diets for 12 weeks dramatically developed hypercholesterolemia. After eight weeks on the diet, the rabbits showed more than a 40-fold increase in the level of plasma cholesterol (1400 mg/dl). After 12 weeks, livers were extracted after the animals were anesthetized by intravenous injection of ketamine hydrochloride (20 mg/kg of body weight). The cholesterol-fed rabbit liver looked abnormal; enlarged and pale, compared to normal liver. Intracellular accumulation of lipids was also obvious in the section of cholesterol-fed liver (date not shown).

Preparation of RNA and differential display of cDNA. Total RNAs from liver were purified by the acid guanidinium thiocyanate–phenol–chloroform extraction method [23]. To remove the chromosomal DNA contamination, the RNAs (50 μg) were treated with 10 U of RNase-free DNase in the presence of an RNase inhibitor, rRNasin (Promega, Madison, WI, USA). Total RNA (2 μg) was reverse-transcribed as described previously [24] using the 3′-primer, that is, 5′-T_{11}VC-3′, where "V" is any one of dGTP, dCTP, and dATP. Synthesized cDNAs (2 μl) were subsequently PCR-amplified with a combination of 25 primer sets (3′-primer: 1 T_{11}VC×5′-primers: 25 arbitrary 10-mers) in the presence of [α-^{35}S]dATP as described previously [14–16].

DNA sequencing and sequence homology search. Differentially expressed cDNAs were cloned into pT7Blue(R) vector (Novagen, Madison, WI, USA) using T4 DNA ligase (TaKaRa, Otsu, Shiga, Japan). The recombinant plasmids were sequenced by the dideoxynucleotide chain-termination method of Sanger et al. [25] using T7 promoter and U19mer primers. The cDNA sequence was compared through the BLAST search program using GBupdate, GenBank, EMBLupdate, and EMBL databases.

Cloning Rab7 cDNA. Primers according to the nucleotide sequences of Rab7 gene from human placenta (GenBank accession no. X93499) and those of the cloned CRGRL-2 were synthesized in Bioneer (Taejon, Korea) (5′-primer: 5′-GGGGTACCTGAAGGATGACCTCTAG GAAGA-3′ and 3′-primer: 5′-CGGGATCCCGCTGTGCTCAACTC TCACTGC-3′). Total RNAs from rabbit liver were reverse-transcribed and the cDNAs were amplified by the PCR as shown above. λgt11 DNAs from human liver cDNA library (Clontech, Palo Alto, CA, USA) purified by ammonium sulfate precipitation method [26] were amplified by the PCR as shown above. The cycling parameters for the amplification were as follows: 1 cycle (95 °C for 10 min) for denaturation and 30 cycles (95 °C for 1 min, 54 °C for 2 min, 72 °C for 1 min) followed by 72 °C for 10 min. The PCR products were cloned into pT7Blue(R) vector and sequenced. Predicted protein sequences were compared with those in the databases for the homology search using the PC/Gene program (IntelliGenetics, Switzerland) and BLAST.

Northern blot analysis. Total RNA (20 μg) was denatured in 2.2 M formaldehyde and 50% formamide at 65 °C for 10 min. The denatured RNA was applied to a 0.9% (w/v) agarose/formaldehyde gel and electrophoresed in 1× MOPS (pH 7.0). The RNA was transferred to a positively charged nylon membrane (Amersham, Cleveland, OH, USA) with 20× SSC (pH 7.0) by the standard capillary transfer method [27]. The nylon filter was UV-cross-linked at 120 mJ/cm^2 using an Ultraviolet Cross-linker (UVP, Cambridge, UK). All probes were [α-^{32}P]dCTP-labeled by the random priming method (Prime-a-Gene Labeling System, Promega, Madison, WI, USA). The filters were hybridized at 42 °C for 16 h in the buffer containing 50% (v/v) formamide, 6× SSC (pH 7.0), 5× Denhardt's solution, 8% (w/w) SDS, denatured salmon sperm DNA (120 μg/ml), and [α-^{32}P]dCTP-labeled probe. Following hybridization, filters were washed with 2× SSC/0.1% SDS and subsequently with 0.2× SSC/0.1% SDS at 42 °C. Filters were exposed to X-ray films at −70 °C with intensifying screens.

Tissue collection and preparation. Hepatic and aortic tissues from hypercholesterolemic and normal rabbits were fixed in 3% formalin and paraffin-embedded. Atherosclerotic lesions were obtained from 10 hospitalized patients in Samsung Medical Center (Seoul, Korea) and Keimyung University School of Medicine (Daegu, Korea).

In situ hybridization. Sections (4 μm) of paraffin-embedded tissues were deparaffinized and dehydrated. The sections were digested with proteinase K (20 μg/ml) at 37 °C for 30 min and postfixed with 0.4% paraformaldehyde at 4 °C for 20 min. Rab7 cDNA was cloned into pSPT18 and pSPT19 vectors (Boehringer–Mannheim, Indianapolis, IN, USA) for the in vitro transcription. Antisense and sense DIG-labeled RNA probes for Rab7 cDNA were produced using a DIG-RNA labeling kit (Boehringer–Mannheim) according to manufacturer's instructions. Prehybridization was conducted to minimize the nonspecific interaction in the mixture of 60% formamide, 5× Denhardt's solution, 4× SSC, 0.1% SDS, 100 μg/ml salmon sperm DNA, 100 μg/ml tRNA, and 10% dextran sulfate for 2 h at 68 °C. Hybridization was carried out with digoxigenin-11-UTP-labeled sense and antisense Rab7 probes for more than 16 h in a humidified chamber followed by an extensive washing with 0.5× SSC three times, 50% formamide/2× SSC at 65 °C, 0.5× SSC two times at 37 °C. The slide was then incubated with avidin-biotinylated alkaline phosphatase (diluted 1:500, Boehringer–Mannheim). The slides were developed with NBT/BCIP (Sigma, St. Louis, MO, USA) to give dark blue products.

Table 1
Multiple alignment of Rab7 sequences from different species

	GTP binding site 1	*Effector binding region*
Rabbit	MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMV	
Human	MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMV	
Dog	MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMV	
Rat	MTSRKKVLLKVIILGDSGVGKTSLMNQYVNKKFSNQYKATIGADFLTKEVMV	
	GTP binding site 2	
Rabbit	DDRLVTMQIWDTAGQERFQSLSVAFYRGADCCVLVFDVTAPNTFKTLDSWRL	
Human	DDRLVTMQIWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRD	
Dog	DDRLVTMQIWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRD	
Rat	DDRLVTMQIWDTAGQERFQSLGVAFYRGADCCVLVFDVTAPNTFKTLDSWRD	
	GTP binding site 3	
Rabbit	EFIQASPRDPENFPFVVLGNKIDLENRQVATKRAQAWSYSKNNIPYFETSAK	
Human	EFIQASPRDPENFPFVVLGNKIDLENRQVATKRAQAWSYSKNNIPYFETSAK	
Dog	EFIQASPRDPENFPFVVLGNKIDLENRQVATKRAQAWSYSKNNIPYFETSAK	
Rat	EFIQASPRDPENFPFVVLGNKIDLENRQVATKRAQAWSYSKNNIPYFETSAK	
		Lipid binding sites
Rabbit	EAINVEQAFQTIARNALKQETEVELYNEFPEPMKLDKNDRAKTSAESCSC	
Human	EAINVEQAFQTIARNALKQETEVELYNEFPEPMKLDKNDRAKASAESCSC	
Dog	EAINVEQAFQTIARNALKQETEVELYNEFPEPMKLDKNDRAKTSAESCSC	
Rat	EAINVEQAFQTIARNALKQETEVELYNEFPEPMKLDKNDRAKASAESCSC	

Sequence of Rab7 from rabbit liver was deduced from the nucleotide sequences determined in this study (GenBank accession No. AF050174) and was compared with the Rab7 sequences from human placenta (X93499), dog kidney (M35522) and rat liver (X12535). Non-matched sequences were shown in black boxes and the functionally important residues were in shaded boxes.

Immunohistochemistry. After deparaffinization followed by dehydration, endogenous peroxidase in the paraffin-embedded tissues (4 μm) was quenched with 3% H_2O_2 in methanol for 30 min. For the antigen retrieval, the sections were heated in 0.01 M citrate buffer (pH 6.0) for 10 min and incubated in 5% goat serum at 37 °C for 30 min. The tissue sections were then allowed to interact with the polyclonal Rab7 antibody (diluted 1:100, Santa Cruz [sc-10767], CA, USA), the anti-CD68 antibody (diluted 1:100, Dako A/S [M0718], Glostrup, Denmark) or anti-α-actin antibody (diluted 1:500, Sigma [A2547], St. Louis, MO, USA) at 4 °C overnight. The Rab7 antibody was generated against the peptides, including from the 158th amino acid to 207th amino acid (Table 1), which contain different sequences from the proteins in Rab family [28]. The section was washed and incubated with the biotinylated secondary antibody (diluted 1:500) at 37 °C for 30 min followed by the incubation in avidin–biotin–peroxidase complex (Vector, Burlingame, CA, USA) at 37 °C for 30 min. The slides were then developed with 3′3′-diaminobenzidene (Sigma, St. Louis, MO, USA) to give brown reaction products followed by counterstaining with hematoxylin.

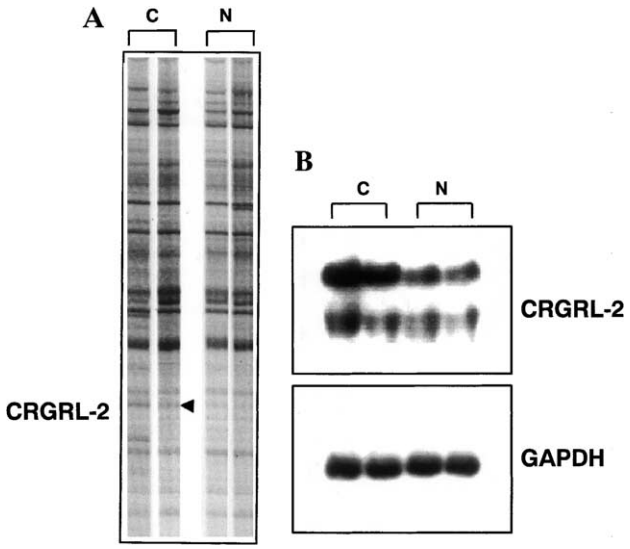

Fig. 1. (A) Representative patterns of differentially displayed hepatic mRNAs obtained from rabbits fed cholesterol-rich (2%, w/w) (c) and normal (*N*) diets using DDRT-PCR. Total hepatic RNAs were isolated from rabbit tissues, reverse-transcribed, and subsequently amplified by a PCR using primer sets: 5′-ACAACGAGG-3′ (10-mer) and 5′-T11VC-3′ (V stands for any one of A, G, or C). Electrophoresis was carried out with the sample on polyacrylamide gel (6%) in duplicate. Arrow-head shows differentially expressed cDNA bands that show differences in their expression upon the high-cholesterol feeding. The indicated cDNA fragment was excised from the gel, re-amplified with the PCR, purified with agarose gel electrophoresis, and subsequently used as a probe for Northern blot hybridization. (B) Differential expression of CRGRL-2 mRNA by high-cholesterol diet was confirmed by Northern blot analysis. Twenty micrograms of total liver RNAs was electrophoresed through 0.9% agarose gel containing formaldehyde, transferred onto nylon membrane, and hybridized with cDNAs [α-^{32}P]-labeled CRGRL-2 (upper panel) and GAPDH (lower panel).

Results and discussion

Differential display of cDNA fragments

Differentially expressed hepatic genes by high-cholesterol diets were monitored by the DDRT-PCR using the total RNAs obtained from liver tissues of the cholesterol-fed rabbits. After the reverse-transcription, the cDNAs were amplified with 25 selected primer sets and displayed on polyacrylamide gels (6%, Fig. 1A). A series of differential display reactions yielded a total of 2500 cDNA bands ranging in size from 100 to 500 bps. Approximately 0.862% (29/2500) of the hepatic mRNAs was responsive to the high-cholesterol diet under the experimental conditions used. All 29 different cDNA bands showing a distinct difference in their thickness compared to the control bands were selected, excised, and re-amplified using the corresponding primer sets. For reverse Northern blot analysis, the reverse-transcribed cDNAs from total RNAs were labeled and allowed to hybridize with the selected cDNAs. Four out of

29 cDNAs were found to be differentially expressed by the high-cholesterol diet, which were later designated as CRGRL-1 (cholesterol-responding gene in rabbit liver) through CRGRL-4. The cDNAs were inserted into pT7Blue(R) vector, transformed, and subjected to colony hybridization to eliminate the possibility that there might be more than one cDNA in each band. The cDNAs were separately labeled with [α-^{32}P]dCTP for Northern blot analysis against the total RNA from hepatic tissues of rabbits fed high-cholesterol and normal diets. Results from the Northern blotting showed that the RNA transcript hybridized with CRGRL-2 is up-regulated in hypercholesterolemic liver compared to the tissue from the control rabbit (Fig. 1B). There were two RNA transcripts (2.5 and 1.5 kb) hybridizing with the ^{32}P-labeled CRGRL-2 cDNA, which later found to be the same RNA transcripts with different lengths of 3′-untranslated regions [29].

Nucleotide sequences of hepatic Rab7 cDNAs

Upon nucleotide sequencing, the CRGRL-2 showed more than 92% homology with the corresponding segment of Rab7 gene from dog, human, rat, and mouse (GenBank accession no. M35522, X93499, X12535, and X89650). Full-length Rab7 cDNA was obtained by RT-PCR from total RNA in rabbit liver (size: 0.7 kb). Nucleotide sequence of rabbit Rab7 gene (as well as its corresponding peptide sequences) was highly conserved: 93% and 92% (98% and 99.5% in protein sequences) in dog and rat, respectively. Human Rab7 cDNA (0.7 kb) was also obtained from human liver cDNA library by PCR. Open Reading Frame regions of Rab7 cDNA from rabbit and human livers (GenBank accession no. AF050174, AF050175) were also highly homologous (>94% and >97% in protein level). There were also identical regions in the sequences; three GTP-binding sites (residue 15–22, 63–67, and 125–128), one effector region (residue 37–45) and two lipid (geranyl–geranyl)-binding sites (residues 205 and 207) (Swiss-Port: accession P51149) that are considered to be functionally important (Table 1).

Localization of Rab7 expression in hypercholesterolemic liver from rabbit

To visualize the location of elevated Rab7 expression in the liver, hepatic tissues from hypercholesterolemic rabbits were subjected to in situ hybridization. Antisense riboprobe against Rab7 cDNA which was prepared by

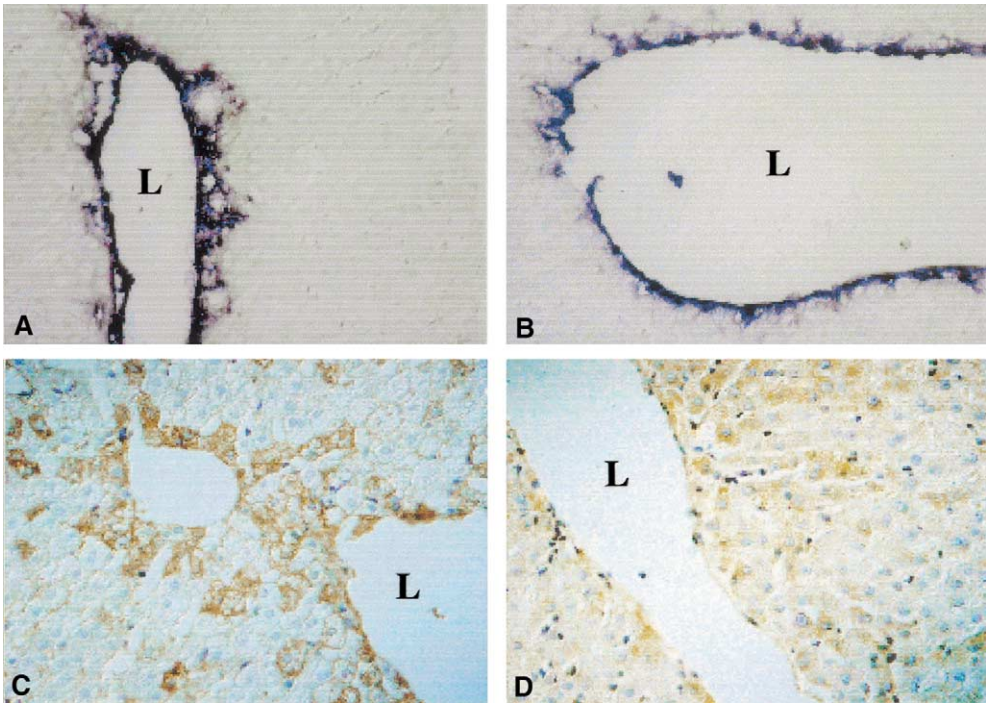

Fig. 2. Histological localization of Rab7 in hypercholesterolemic rabbit livers. New Zealand white rabbits (aged 6 weeks, 2.5–3 kg) were fed 2% (w/w) cholesterol diet for 12 weeks. Liver tissues obtained from the rabbits fed the cholesterol-rich diet (A and C) or a normal lab chow (B and D) were fixed with 3% (v/v) formalin and embedded in paraffin. For in situ hybridization, the DIG-labeled antisense Rab7 probe was allowed to hybridize with sections of liver from the rabbits (panels A and B). To visualize the Rab7 in a protein level, the sections were incubated with polyclonal Rab7 antibody at 4 °C overnight followed by an application to the avidin–biotin–horseradish peroxidase system (Vector, Burlingame, CA, USA) (panels C and D). The sections were subsequently counterstained with hematoxylin to visualize nuclei. L: Lumen; Magnification: 400×; Microscope: Axioskop from Zeiss (Germany).

the in vitro transcription in the presence of digoxigenin-11-UTP was used for the in situ hybridization. In the hypercholesterolemic rabbit liver, the Rab7 was heavily expressed in endothelial cells and hepatocytes near the central veins, compared to the tissues from rabbit fed an ordinary laboratory chow (Fig. 2A and B). In addition, the expression of Rab7 in the liver tissue was also visualized by the immunohistochemistry using anti-Rab7 antibody raised with a specific region of the protein. As shown in Fig. 2 (panels C and D), the expression of Rab7 was elevated and concentrated in the region of central vein in the hypercholesterolemic liver, compared to a lower level of expression with a scattered distribution in the normal tissue. It has been previously shown that the Rab7 is expressed normally in the endothelial cells 4–7 times more than in the parenchymal cells in the liver [30]. It was also shown that the cholesterol uptake in the hepatic endothelia in the diet-induced hypercholesterolaemic rabbit was 5-fold higher than that of rabbit on the normal diet [31]. When the rabbit was on the high-cholesterol diet, as shown in this study, the expression of Rab7 was further increased in the region of endothelia and hepatocytes close to the central vein. Those cells surrounding central veins in the hypercholeserolemic liver appear to engulf an excess amount of exogenous cholesterol and potentially turn into lipid-accumulating cells.

Expression of Rab7 in atherosclerotic tissues

To examine a potential relationship between the Rab7 and the atherogenesis, expressional patterns of the gene were monitored in the aortic tissues from the hypercholesterolemic rabbit and human atherosclerotic plaques from hospitalized patients by in situ hybridization and immunohistochemistry. The Rab7 mRNA was intensively expressed in the intima of early atheroscle-

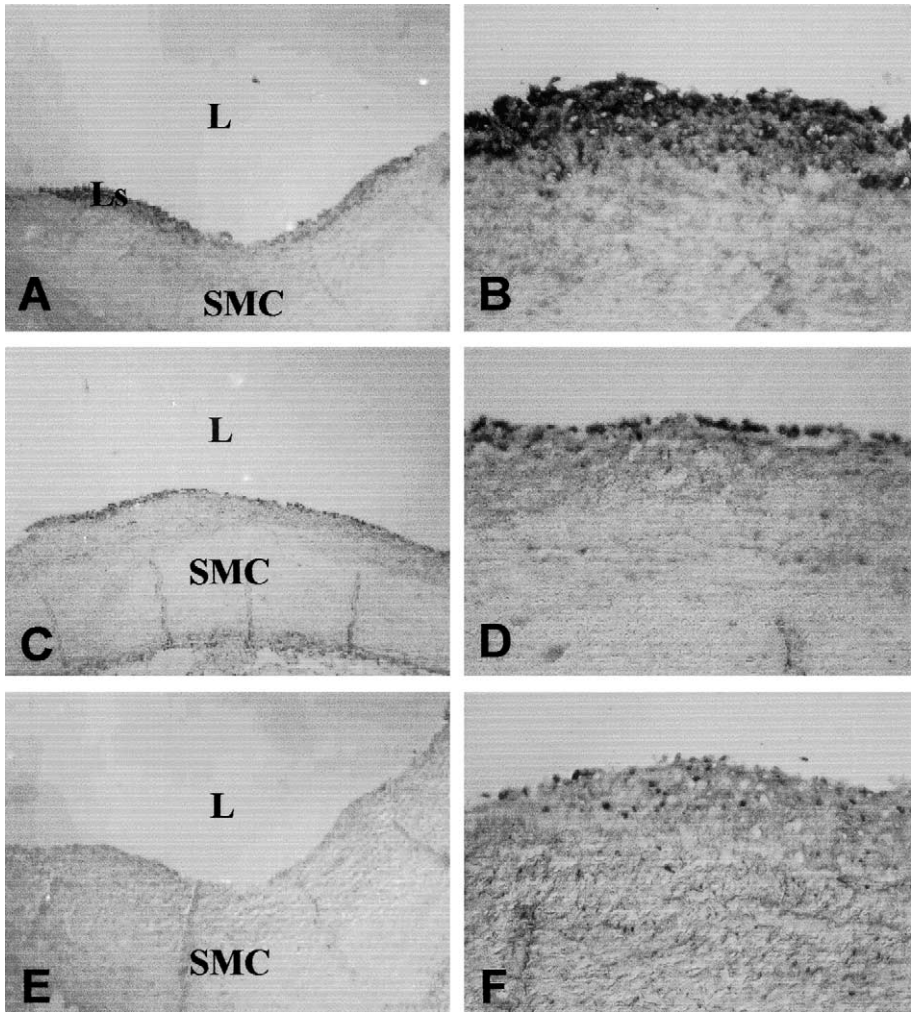

Fig. 3. Elevated expression of Rab7 in the aortas of high cholesterol-fed rabbits. Sections of A, B, E, and F were from arteries of hypercholesterolemic rabbits. Those of C and D were from arteries of normal rabbits. A DIG-labeled antisense Rab7 probe was hybridized with the sections (panels A–D), as shown in Fig. 2. A DIG-labeled sense Rab7 probe was used (panels E and F) as a negative control. L: Lumen; Ls: Lesions; SMC: Smooth muscle cells. Magnification: 100× for panels A, C, and E; 400× for panels B, D, and F.

rotic lesions (Fig. 3A and B), whereas a minimal level of the Rab7 was expressed in the normal aortic endothelial cells (Fig. 3C and D). In the protein level depicted by the immunostaining, the Rab7 was heavily expressed in the intima of early atherosclerotic lesions from hypercholesterolemic rabbit (Fig. 4A and B). On the other hand, a minimal level of Rab7 was expressed in the rabbit aortas, which were free of atherosclerotic lesion (Fig. 4C and D).

In human atherosclerotic plaques, the elevated expression of Rab7 was observed in the region of intima upon staining a section of plaque with the anti-Rab7 antibody (Fig. 5A, B, and F). When the tissue was immunostained with the antibody against a macrophage-specific protein, CD68, a similar region was stained (Fig. 5D and G). This suggests that the elevated expression of Rab7 may be confined to the macrophage-rich region. When the tissue was subjected to the in situ hybridization, the Rab7 mRNA was hybridized with digoxigenine-labeled antisense riboprobe in the region of intima as well as lacerated and partial dissected atherosclerotic tissues (Fig. 5E). Those regions also coincided where the CD68 is located (Fig. 5G).

Atherosclerotic plaques were generally believed to originate from many cells including monocytes/macrophages, blood vessel endothelial, and smooth muscle cells. In the studies of expressional pattern of Rab7 using cell lines in our laboratory, the monocytic THP-1 cells showed the highest level of Rab7 expression, compared to HUVEC (endothelium) and A10 (smooth muscle cells) cells (unpublished data). The reason for the elevated Rab7 expression in macrophage-containing tissue and its relationship with atherogenesis are unknown. However, there is a line of evidence to show that many kinds of atherogenic factors may play the role [32,33]. According to the recent report on the functional truncation of Rab7 by the microinjection of Rab GDP-dissociation inhibitor (Rab-GDI) into the LDL-fed cells, the distribution of Rab7 has been altered to the cytosol in a diffused form from a punctate distribution in the perinuclear region with a normal Rab7 [34]. This indicates that an alteration in the Rab7 may lead to a

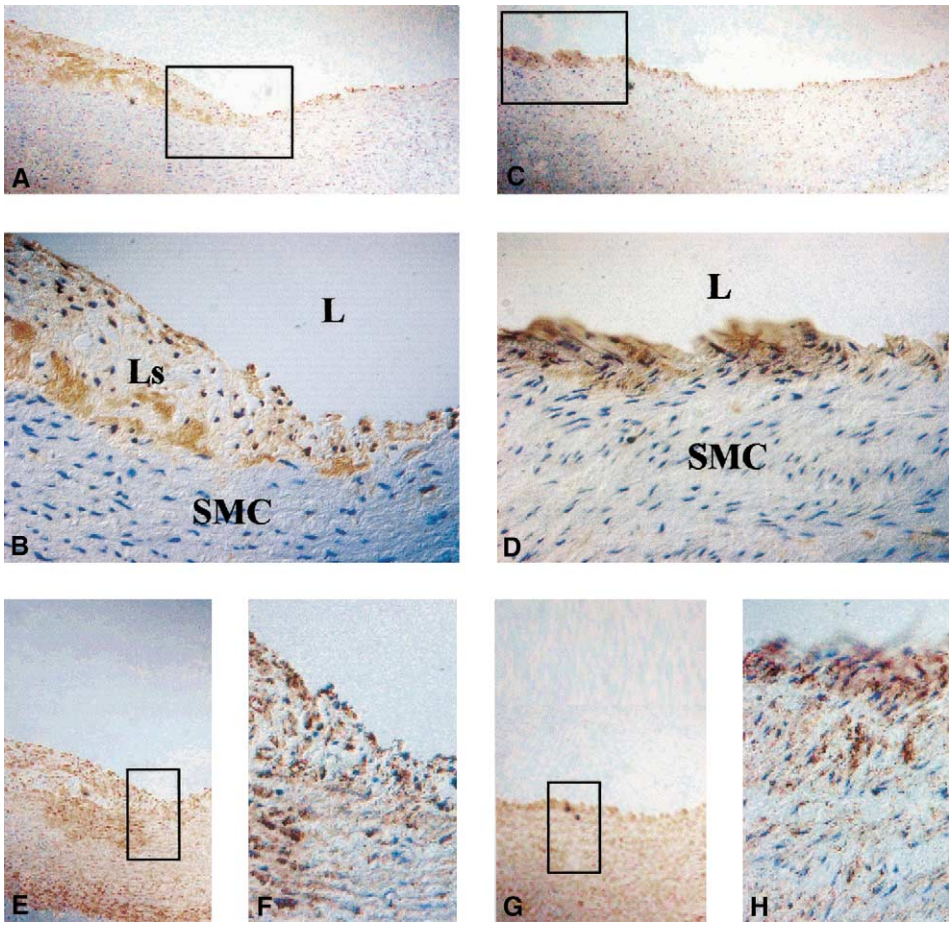

Fig. 4. Immunolocalization of Rab7 in atherosclerotic lesions of aortas from high cholesterol-fed rabbits. Sections of A, B, E, and F were from arteries of hypercholesterolemic rabbits. Those of C, D, G, and H were from normal rabbits. Serial sections of arteries were incubated with polyclonal Rab7 antibody (panels A–D) and with anti-α-actin antibody (panels E–H), a specific marker of smooth muscle. All immunostained sections were counterstained with hematoxylin to visualize nuclei. The box indicates the enlarged region. L: lumen; Ls: lesions; SMC: smooth muscle cells. Magnification: 100× for panels A, C, E, and G; 400× for panels B, D, F, and H.

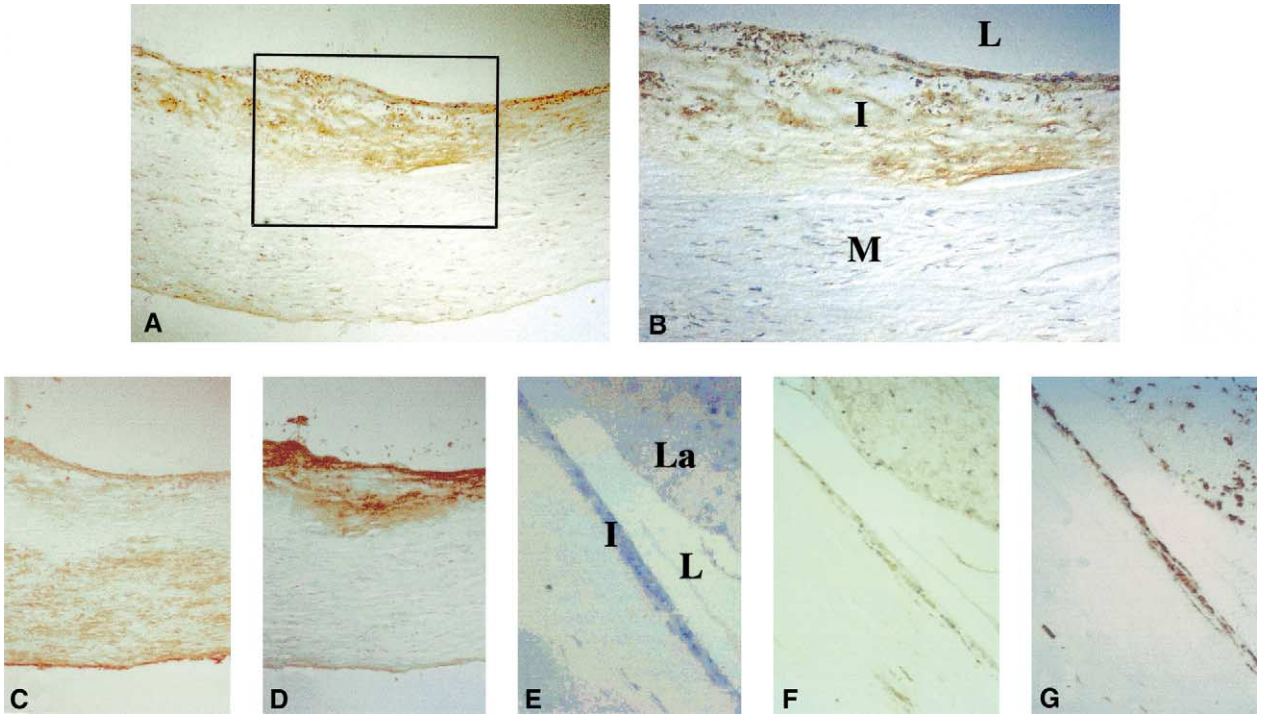

Fig. 5. Expression of Rab7 in human atherosclerotic plaques. The immunohistochemistry and in situ hybridization were conducted as shown in Fig. 2. Serial sections (4 μm in thickness) were allowed to react with polyclonal Rab7 antibody (A, B, and F), anti-α-actin antibody to visualize the smooth muscle (C), anti-CD68 antibody for macrophage (D and G). Result from in situ hybridization with a DIG-UTP labeled antisense Rab7 probe is shown in panel E. The tissues shown in panels E–G were a part of tissue in the blood vessel generated by a laceration and partial dissection of the atherosclerotic tissue. The box indicates the enlarged region. All panels of immunohistochemistry were counterstained with hematoxylin to visualize nuclei. L: Luman; I: Intima; M: Media; La: Lacerated tissue. Magnification: 100× for panels A, C, and D; 200× for panels B, E, F, and G.

profound change in the cholesterol trafficking in the cell, which may result in the malfunction of the cells. Normally, the Rab7 is localized in the late-endosomal fractions containing cholesterol esters, tryglycerides, and phospholipids [35]. However, the oxidized LDL, a major atherogenic factor, contains many components that may affect the function of Rab7, such as oxidized form of apolipoprotein B, oxysterols, and lysophosphatidylcholine, etc. When the macrophages engulf a large quantity of oxidized LDL, this may induce a differential expression of genes relevant to the atherogenesis [36–38], such as Rab7. With an altered expression of the genes by an atherogenic factor such as excess cholesterol, the Rab7-mediated transition of late-endosome to lysosome could be altered during the endocytic process of LDL or modified LDL. Taking these into considerations together with the altered expression of Rab7 by the cholesterol feeding, the Rab7 could be an important parameter in the process of atherogenesis.

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