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Species differences of macrophage very low-density-lipoprotein (VLDL) receptor protein expression

Sadao Takahashi [a,b,*], Takashi Ito [c], Yasuo Zenimaru [a], Jinya Suzuki [a], Isamu Miyamori [a], Masao Takahashi [d], Masafumi Takahashi [e], Takafumi Ishida [f], Tatsuro Ishida [g], Ken-ichi Hirata [g], Tokuo T. Yamamoto [h], Tadao Iwasaki [i], Hiroaki Hattori [i], Masashi Shiomi [c,j]

[a] Third Department of Internal Medicine, University of Fukui, Faculty of Medical Science, Japan
[b] Research and Education Program for Life Science, University of Fukui, Faculty of Medical Science, Japan
[c] Institute for Experimental Animals, Kobe University Graduate School of Medicine, Japan
[d] Department of Cardiovascular Surgery, Hiratsuka Kyosai Hospital, Japan
[e] Division of Bioimaging Sciences, Center for Molecular Medicine, Jichi Medical University, Japan
[f] Department of Cardiovascular Medicine, Hiroshima University Graduate School of Biomedical Sciences, Japan
[g] Division of Cardiovascular Medicine, Kobe University Graduate School of Medicine, Japan
[h] Center for Advanced Genome Research, Institute of Development, Aging, and Cancer, Tohoku University, Japan
[i] Department of Advanced Medical Technology and Development, BML, Inc., Japan
[j] Section of Animal Models for Cardiovascular Diseases, Kobe University Graduate School of Medicine, Japan

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ABSTRACT

Triglyceride-rich lipoproteins (TGRLs) and low-density-lipoprotein (LDL) cholesterol are independent risk factors for coronary artery disease. We have previously proposed that the very low-density-lipoprotein (VLDL) receptor is one of the receptors required for foam cell formation by TGRLs in human macrophages. However, the VLDL receptor proteins have not been detected in atherosclerotic lesions of several animal models. Here we showed no VLDL receptor protein was detected in mouse macrophage cell lines (Raw264.7 and J774.2) or in mouse peritoneal macrophages in vitro. Furthermore, no VLDL receptor protein was detected in macrophages in atherosclerotic lesions of chow-fed apolipoprotein E-deficient or cholesterol-fed LDL receptor-deficient mice in vivo. In contrast, macrophage VLDL receptor protein was clearly detected in human macrophages in vitro and in atherosclerotic lesions in myocardial infarction-prone Watanabe-heritable hyperlipidemic (WHHLMI) rabbits in vivo. There are species differences in the localization of VLDL receptor protein in vitro and in vivo. Since VLDL receptor is expressed on macrophages in atheromatous plaques of both rabbit and human but not in mouse models, the mechanisms of atherogenesis and/or growth of atherosclerotic lesions in mouse models may be partly different from those of humans and rabbits.

1. Introduction

The role of low-density-lipoprotein cholesterol (LDL-C) in the development and progression of atherosclerosis has been established in humans [1]. Both qualitative and quantitative abnormalities in circulating triglyceride-rich lipoproteins (TGRLs) may be key factors in the development of human coronary artery diseases (CADs) [2]. The development of macrophage foam cells that contain massive amounts of cholesterol ester is a hallmark of both early and advanced atherosclerotic lesions [3]. There are two major lipoproteins that contribute to macrophage foam cell formation. One is LDL and the other is VLDL. LDL particles that infiltrate into arterial subendothelial regions are oxidized and oxidized LDL (oxLDL) particles are taken up by macrophages through scavenger receptors. VLDL particles without oxidative modification are taken by macrophages through VLDL receptors and remnant receptors in human macrophage cell lines (phorbol-12-myristate-13-acetate: PMA-treated THP-1 monocytic leukemia cells and HL-60 cells) and in human monocyte-derived macrophages [4,5]. In mouse peritoneal macrophages, however, LDL-receptor-related protein-1 (LRP-1) and the LDL receptor that recognizes TGRLs are candidate receptors for mediation of macrophage foam cell formation [6,7].

We were the first to clone and characterize VLDL receptor cDNAs from rabbit heart and human THP-1 cells [8,9]. The VLDL

Abbreviations: TGRLs, triglyceride-rich lipoproteins; VLDL, very low-density lipoprotein; LDL, low-density-lipoprotein; WHHLMI, myocardial infarction-prone Watanabe-heritable hyperlipidemic; apo, apolipoprotein; CADs, coronary artery diseases.

* Corresponding author at: Third Department of Internal Medicine, University of Fukui, Faculty of Medical Science, Japan. Fax: +81 776 61 8111.
E-mail address: sadaost@u-fukui.ac.jp (S. Takahashi).

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receptor is abundantly expressed in tissues that are active in fatty acid metabolism (heart, skeletal muscle and fat) as well as in brain and macrophages. The ligand specificity of the VLDL receptor is different from that of the LDL receptor. The VLDL receptor only binds to apolipoprotein (apo) E-containing particles such as VLDL and intermediate-density lipoprotein (IDL) obtained from Watanabe-heritable hyperlipidemic (WHHL) rabbits as well as to β-VLDL obtained from cholesterol-fed rabbits. These findings indicated that the VLDL receptor is a lipoprotein receptor for TGRLs, but not for LDL. ApoE and LPL, which are secreted by heart, skeletal muscle, fat and macrophages, accelerate the binding of TGRLs to the VLDL receptor [10]. We proposed that the VLDL receptor functions as a peripheral lipoprotein receptor in tissues active in fatty acid metabolism even though it is true that the VLDL receptor and apoE receptor 2 (ApoER2) are reelin receptors in brain [11,12].

Previous studies have demonstrated that the VLDL receptor protein and mRNA are detected in human atherosclerotic lesions [13,14]. Although the expression of VLDL receptor mRNA in atherosclerotic lesions of rabbits was observed [15,16], no studies have shown VLDL receptor protein in atherosclerotic lesions of rabbits and mice. Detection of VLDL receptor protein in atherosclerotic lesions is important in understanding the mechanisms of atherogenesis and growth of atherosclerotic lesions. We were fortunate enough to obtain a rabbit polyclonal antibody that reacts with human, rabbit, rat and mouse heart VLDL receptor proteins. Using this antibody, we found definite species differences in macrophage VLDL receptor protein expression.

2. Materials and methods

2.1. Reagents

Phorbol-12-myristate-13-acetate (PMA) was purchased from Wako Pure Chemical Industries (Osaka, Japan). RPMI-1640, DMEM

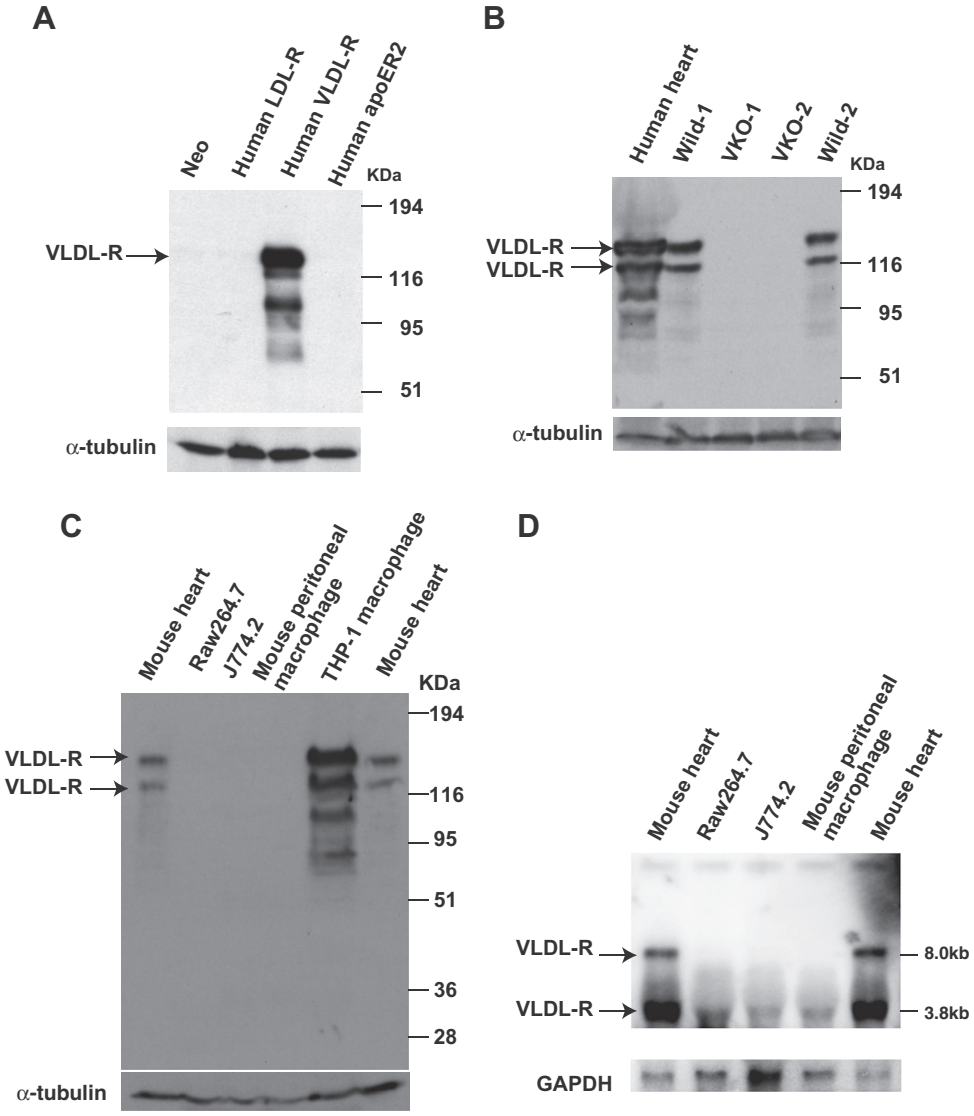

Fig. 1. Characterization of the rabbit polyclonal antibody (VR2) and in vitro assay of VLDL receptor expression in mice and humans. (A) The pSV2-neo plasmid encoding the full-length human LDL receptor (LDL-R), human VLDL receptor (VLDL-R), human apoE receptor 2 (apoER2) cDNA or control pSV2-neo alone (Neo) was transfected into a mutant Chinese hamster ovary cell line lacking LDL receptors (ldlA-7 cells) and G418 selection was performed. Cell lysates were analyzed by Western blot using VR2. (B) Heart tissues from human after Batista operation, two wild-type (Wild-1/-2) and two VLDL-receptor knockout (VKO-1/-2) mice were lysed and analyzed by Western blot using VR2. The higher molecular band is the type 1 VLDL receptor protein and lower band is the mixture of proteins of type 1 VLDL receptor precursor and type 2 VLDL receptor. For Fig. 1A and B, α-tubulin was blotted as a loading control. (C) Cell lysates of the mouse macrophage cell lines (Raw264.7 and J774.2), mouse peritoneal macrophages and PMA-induced human THP-1 macrophage cells were analyzed by Western blot using the VR2 antibody. Mouse heart from wild-type mice was used as a positive control for VLDL receptor proteins. (D) VLDL receptor and GAPDH mRNA expression in the indicated cells and tissues was analyzed by Northern blot analysis.