



Low Density Lipoprotein Receptor-related Protein 2 Expression and Function in Cultured Astrocytes and Microglia

Katsura Takano-Kawabe¹ · Kazuyuki Matoba¹ · Yoichi Nakamura¹ · Mitsuaki Moriyama¹

Received: 26 July 2023 / Revised: 23 August 2023 / Accepted: 28 August 2023 / Published online: 13 September 2023
© The Author(s), under exclusive licence to Springer Science+Business Media, LLC, part of Springer Nature 2023

Abstract

Activation of glial cells, astrocytes and microglia, has been observed in neurodegenerative diseases including Alzheimer's disease (AD). Amyloid β ($A\beta$), which is aggregated and the aggregation is detected as characteristic pathology in AD brain, is known to be produced by neurons and to activate glial cells. Clearance of $A\beta$ from the brain via active transport system is important to prevent the accumulation and aggregation. Low density lipoprotein receptor-related protein 2 (LRP2/megalin) is an $A\beta$ transporter. However, expression and contribution of LRP2 in astrocytes and microglia remain to be clarified. In the present study, we examined the expression of LRP2 and its roles in cultured astrocytes prepared from rat embryonic brain cortex and mouse microglial cell line BV-2. Both cultured rat astrocytes and BV-2 cells expressed LRP2 mRNA detected by RT-PCR. When lipopolysaccharide (LPS) or all-trans retinoic acid (ATRA) were added to BV-2 cells, LRP2 mRNA expression and uptake of microbeads, $A\beta$ and insulin were increased. On the other hand, LPS decreased LRP2 expression and uptake of $A\beta$ and insulin in cultured astrocytes. Knockdown of LRP2 using siRNA attenuated the LPS- or ATRA-increased uptake of microbeads, $A\beta$ and insulin in BV-2 cells. These results suggest that LRP2 was expressed in both astrocytes and microglia and might be involved in endocytosis activities. Adequate control of LRP2 expression and function in astrocytes and microglia might regulate $A\beta$ and insulin levels in brain and would be a potential target in AD pathology.

Keywords LRP2 · Astrocyte · Microglia · Amyloid β · Insulin

Abbreviations

$A\beta$	Amyloid beta	iNOS	Inducible nitric oxide synthase
AD	Alzheimer's disease	LDL	Low density lipoprotein
ApoE	Apolipoprotein E	LDLR	Low density lipoprotein receptor
APP	Amyloid precursor protein	LPS	Lipopolysaccharide
ATRA	All-trans retinoic acid	LRP	Low density lipoprotein receptor-related protein
BBB	Blood brain barrier	NF- κ B	Nuclear factor-kappa B
CNS	Central nervous system	NO	Nitric oxide
DMEM	Dulbecco's modified Eagle medium	PBS	Phosphate buffered saline
FBS	Fetal bovine serum	RA	Retinoic acid
FITC	Fluorescein isothiocyanate	RAGE	Receptor of advanced glycation end product
GFAP	Glial fibrillary acidic protein	RAR	Retinoic acid receptor
gp	Glycoprotein	RARE	Retinoic acid response element
		ROS	Reactive oxygen species
		RXR	Retinoid receptor
		TLR	Toll-like receptor

✉ Katsura Takano-Kawabe
takano@omu.ac.jp

¹ Laboratory of Integrative Physiology in Veterinary Sciences, Osaka Metropolitan University, 1-58, Rinku-Ourai Kita, Izumisano, Osaka 598-8531, Japan

Introduction

Low density lipoprotein (LDL) receptor-related protein (LRP) is a single transmembrane glycoprotein (gp) belonging to the LDL receptor family, and generally recognized as cell surface endocytic receptors [1, 2]. LRPs bind and internalize extracellular ligands for degradation in lysosomes [3]. The LDL receptor (LDLR) family consists of more than 11 receptors that function in receptor-mediated endocytosis and cellular signaling [4]. Among them, the most important in central nervous system (CNS) are LRP1 and LRP2, and they are multifunctional cell surface receptors that are widely expressed in several tissues including neurons and astrocytes [3].

LRP1, also known as CD91 or $\alpha 2$ macroglobulin receptor, is a multifunctional scavenger and signaling receptor that belongs to the LRP family [5, 6]. LRP1 is a massive protein (600 kDa) that is proteolytically nicked during biosynthesis to give two stably associated polypeptides: an 85-kDa membrane-spanning C-terminal fragment and a 515-kDa extracellular N-terminal chain. LRP1 binds more than 30 ligands extracellularly, including apolipoprotein E (ApoE), blood coagulation factors, amyloid β (A β), and prion protein [3]. LRP1 is abundantly expressed in neurons [7], astrocytes [8], and microglia [9]. LRP1 is also expressed in the CNS including vascular cells such as brain endothelial cells and pericytes, and in the choroid plexus of the blood brain barrier (BBB) [4]. LRP1 in neurons plays fundamental roles to uptake cholesterol and fatty acids required for synapse formation [10], and interacts with amyloid precursor protein (APP) and regulates proteolytic processing to increase the production of A β peptide [11].

LRP2 was originally identified as the major antigen in Heymann nephritis, a rat model of nephropathy, and named gp330 [12]. LRP2, also called megalin, is structurally very similar to LRP1 and many common ligands were known [3]. LRP2 was reported to be expressed in neurons [13] and astrocytes [14]. In astrocytes, LRP2 is required for albumin binding and internalization into astrocytes including synthesis of neurotrophic factors by the neighboring neurons [15]. However, other reports showed that LRP2 mRNA could not be detected in human cultured astrocytes [16], mouse astrocytes [17], and adult rat brain [18]. Therefore, LRP2 expression in astrocytes needs more studies. Moreover, there were few studies for LRP2 expression in microglia.

Alzheimer's disease (AD) is a progressive neurodegenerative disease characterized by the progressive declines of memory and cognitive functions, and changes in behavior and personality [19]. The most accepted hypothesis for the mechanism of brain injury in AD is the "amyloid cascade", comprising amyloid accumulation in the brain, the formation of toxic oligomeric and intermediate forms of

A β peptides, amyloid plaques, inflammation and the induction of neurofibrillary tangles [20]. There is accumulation of A β in both the normal aging brain and the AD brain, and defective A β clearance rather than increased A β production is thought to be related to the accumulation [21]. Clearance of A β from the brain occurs via active transport at the interfaces separating the CNS from the peripheral circulation. Through the BBB, LRP1 and LRP2 facilitate the clearance of the A β peptide that is produced by amyloidogenic processing of APP, which can form complexes with different LRPs' ligands such as ApoE [22, 23]. In the brain cells, astrocytes and microglia can uptake A β for eliminating and contribute to A β clearance [24].

Several lines of evidence have implicated various LRPs and LRP ligands in the pathogenesis of AD [25]. LRP1 and LRP2 also might be linked to AD. LRP1 antisense or endothelial-specific LRP2 null in BBB caused AD-like impaired cognition in mice [26, 27]. On the other hand, it was reported that LRP1 increased but LRP2 decreased in choroid plexus epithelium in aged rat [28]. Further, in brain tissues from patients with AD, LRP2 expression has been immunohistochemically detected in neurons, even being up-regulated in damaged neurons [13]. Therefore, LRP1 and LRP2 might have different speculative effects in the brain function and AD pathology. LRP1 have been well investigated whereas LRP2 is less studied. Thus, we assessed LRP2 expression in astrocytes and microglia in the present study.

LRP1 and LRP2 can bind to A β and facilitate the clearance of the A β as described above. In addition, LRP1 and LRP2 can bind to insulin and endocytose, but none is known about the roles of LRP1 and LRP2 in insulin transport into brain [29]. Insulin elongates neuronal axon, potentiates protein synthesis in neurons, and increases synapse formation [30]. It is known that insulin signaling is important in normal neuronal and brain functions and that neurons produce and release insulin by exocytotic mechanism and use it by themselves [30]. In AD pathology, A β has been reported to impair insulin signaling in neurons and increase phosphorylation of tau protein causing neurofibrillary tangles [31, 32]. Furthermore, it was reported that insulin concentration in cerebrospinal fluid decreased in severe AD patients [33] and that A β reduced insulin synthesis in cultured neurons [30]. In the previous study, we found that insulin was expressed also in astrocytes and released to extracellular medium [34]. On the other hand, it was reported that brain insulin was partly derived from pancreatic β cells to be permeated BBB [35, 36], but the involvement of LRP2 in the process was not identified.

In the present study, we assessed the expression of LRP2 and its function in endocytosis activity of microbeads, A β and insulin using cultured astrocytes and mouse microglial cell line BV-2. Further, we also investigated the effects

of lipopolysaccharide (LPS) and all-trans retinoic acid (ATRA) on LRP2 expression and the endocytosis activity. LPS is a commonly used activator of astrocytes and microglia in experiments [37, 38]. ATRA is known to induce proliferation and differentiation of various cells [39, 40] and decrease the activation of astrocytes and microglia [41].

Experimental Procedures

Materials

Deoxyribonuclease I (DNase I), trypsin, anti-A β antibody, anti-gial fibrillary acidic protein (GFAP) antibody, and fluorescein isothiocyanate (FITC)-conjugated insulin were purchased from Sigma Chemical Co. (St. Louis, MA, USA). Dulbecco's modified Eagle medium (DMEM) and horse serum were from Gibco BRL (Grand Island, NY, USA). Fetal bovine serum (FBS) was from GE Healthcare Inc. (Little Chalfont, Bucks, UK). A β_{1-42} was from Peptide Institute, Inc. (Osaka, Japan). Rat and mouse LRP2 primers and 18S rRNA primer were from Eurofins Genomics Inc. (Tokyo, Japan). Control siRNA and LRP2 siRNA were from Santa Cruz Biotechnology Inc. (Dallas, TX, USA). HilyMax® was from Dojindo (Kumamoto, Japan). FITC-conjugated anti-mouse IgG antibody and rhodamine-conjugated anti-rabbit IgG antibody (Jackson ImmunoResearch, PA, USA).

Preparation of Astrocytes Culture

This study was carried out in compliance with the Guideline for Animal Experimentation at Osaka Prefecture University, with an effort to minimize the number of animals used and their suffering. Astrocytes were prepared as described previously [37]. In brief, cortex from 20-day-old embryos, which were taken out from pregnant Wistar rats deeply anesthetized, were cleared of meninges, cut into about 1-mm³ blocks, and treated with 0.25% trypsin in Ca²⁺, Mg²⁺-free phosphate-buffered saline (PBS) containing 5.5 mM glucose for 20 min at 37°C with gentle shaking. An equal volume of horse serum supplemented with 0.1 mg/mL of DNase I was added to the medium to inactivate the trypsin. Then, the tissues were centrifuged at 350 × *g* for 5 min. The tissue sediments were triturated through a pipette with DMEM containing 10% FBS, 50,000 units/L penicillin and 100 mg/L streptomycin. After filtering cell suspensions through a lens-cleaning paper (Fujifilm Co., Tokyo, Japan), the cells were plated on polyethyleneimine-coated 100-mm diameter plastic dishes (Iwaki, Asahi Glass Co., Tokyo, Japan) at a density of 0.8–1.3 × 10⁵ cells/cm². Cultures were maintained in a humidified atmosphere of 5% CO₂ and 95%

air at 37°C with changing medium every 3 days. After one week, astrocytes were replated to remove neurons. On days 14–21, they were replated onto adequate plates or dishes using an ordinary trypsin-treatment technique at a density of 0.8–1.3 × 10⁵ cells/cm², and stabilized for 1 day, then we used for experiments.

More than 90% of the cells were immunoreactively positive to an astrocyte marker, glial fibrillary acidic protein (GFAP), using the antibody (Biosensis Pty Ltd., Thebarton, Australia) and FITC-conjugated anti-mouse IgG antibody. Less than 10% of the cells were positive to a microglial marker, Iba-1; using the antibody (Wako) and rhodamine-conjugated anti-rabbit IgG antibody.

BV-2 Cell Culture

Mouse microglial cell line BV-2 cells were grown and maintained in DMEM supplemented with 10% FBS and penicillin (50,000 units/L)/streptomycin (100 mg/L) at 37°C in a humidified incubator under 5% CO₂. Cells were subcultured once a week. They were replated onto adequate plates or dishes using an ordinary trypsin-treatment technique at a density of 0.8–1.3 × 10⁵ cells/cm², and stabilized for 1 day, then we used for experiments. BV-2 cells were kindly provided from Laboratory of Molecular Pharmacology, Kanazawa University Graduate School.

Realtime Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

For realtime RT-PCR assays, the cells were replated onto 12-well plate (Iwaki, Japan) and stabilized for 1 day in medium containing 10% FBS. And then, the cells were incubated for 24 h and stimulated for 24 h in serum-free medium. Total RNA was isolated using FavorPrep™ Tissue Total RNA Purification Mini Kit (Favorgen, Taiwan). Complementary DNA was prepared using Omniscript Reverse Transcription kit (Qiagen, Germany) according to the manufacturer's protocol. PCR experiments were done using SYBR® Green Realtime PCR Master Mix (Toyobo, Japan) for LRP2 and 18S rRNA. Table 1 shows the list of the sequence of the primers (Eurofins Genomics, Japan) and cycling protocols used in this study. Detection of the fluorescent product was carried out at the end of the extension period. To compare mRNA levels among samples, mRNA for each gene of interest was normalized to the expression of a housekeeping gene, 18S rRNA using the comparative Ct method. The changes in the levels of each PCR product were calculated as ratio of control.

Table 1 PCR primers for LRP2

	primer	Denaturation (15 s)	Annealing+Extension (1 min)
Rat LRP2	forward	5'-GCA GAG ATG GAC AGT GAG GT-3'	95°C
	reverse	5'-GCT GGC GAG GCT ATA CG-3'	62°C
Mouse LRP2	forward	5'-GCA AGT GTT CTA GCG GCT AC-3'	95°C
	reverse	5'-TGG GCA GAG AGG GTA AAA GG-3'	60°C
18S rRNA	forward	5'-GTA ACC CGT TGA ACC CCA -3'	95°C
	reverse	5'-GGT AGG TTA GCC ATC ATC GCT-3'	60°C

Immunostaining

The cells were fixed by 4% paraformaldehyde for 30 min at room temperature, and permeabilized with 0.5% Triton X-100 in PBS for 30 min at room temperature. After blocking with 1% bovine serum albumin (BSA) and 4% normal goat serum in PBS for 30 min, the cells were incubated with antibodies against A β (1:100), and GFAP (1:750) in blocking buffer at 4°C overnight followed by an incubation with FITC-conjugated anti-mouse IgG antibody (1:50) or rhodamine-conjugated anti-rabbit IgG antibody (1:50) at room temperature for 3 h. Then, nuclear stain was performed using DAPI for 10 min, and the cells were mounted with SlowFade® Diamond (Molecular Probes). To visualize fluorescent immunostaining, Olympus IX70 microscope was used.

Endocytosis Assay

Endocytosis was assessed by uptake of carboxylate modified fluorescent microspheres (diameter = 1 μ m) (Molecular Probes, OR, U.S.A.). 10 μ L of fluorescent microspheres was mixed with 5 μ L FBS, and diluted 100 times in medium containing 10% FBS (microbeads solution). After the BV-2 cells were stimulated, 50 μ L/well of microbeads solution (final concentration; 0.013% solid) was added to the cells and incubated for 1 h. Then the cells were washed by DMEM three times to remove non-endocytosed beads, and observed under a fluorescent microscopy (Olympus IX70). The photographs of phase contrast and fluorescent images were taken at each same site. More than 20 endocytotic cells in each photograph were picked up to measure cellular fluorescence intensity by Image J software, and average of the intensity was evaluated.

Uptake of A β or Insulin

Uptake of A β : A β _{1–42} was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1 mM as a stock solution and kept at –80°C before using. The serum-free DMEM was added with A β stock solution at the concentration of 10 μ M and incubated for 14 days at 37°C (aggregated A β), then used for A β uptake. After the cell stimulation, 0.1 μ M A β was added to the cells and incubated for 6 h. Then the cells were washed by PBS and immunostained as described above. In cultured astrocytes, the total numbers of GFAP-positive cells and A β -positive cells were counted and the ratio of A β and GFAP double-positive cells per GFAP-positive cells was calculated. In BV-2 cells, because almost every cells endocytosed A β , more than 20 cells in each photograph were picked up to measure cellular fluorescence intensity by Image J software, and average of the intensity was evaluated.

Uptake of insulin: After stimulation, 50 nM FITC-conjugated insulin was added to the cells and incubated for 30 min. Then the cells were washed by PBS, and fixed by 4% paraformaldehyde. After washing by PBS, the cells were observed under a fluorescent microscope (Olympus IX70). Total cells and FITC fluorescence superposed cells were counted.

Transfection

For knockdown of LRP2 expression, siRNA was transfected using HilyMax® (Dojindo) according to the manufactures' protocol. In brief, 20 pmol LRP2 siRNA or negative control (CTL) siRNA was mixed with serum-free DMEM, and HilyMax solution was added and incubated for 15 min at room temperature to form siRNA-HilyMax complex.

BV-2 cells were replated onto 24 well plate (Iwaki) and stabilized for 1 day. siRNA-HilyMax complex was added to the cells in serum-free DMEM and incubated for 24 h. And then, the stimulation for 24 h in each experiment was performed.

Data Analysis

For statistical analysis of the data, one-way ANOVA followed by Tukey-Kramer or Dunnet multiple comparison procedure or Student's *t* test was used. Differences between treatments were considered statistically significant when $p < 0.05$.

Results

Changes of LRP2 mRNA Expression in Cultured Astrocytes

To assess the changes of LRP2 expression with various stimulation in cultured astrocytes, the cells were incubated with LPS (0.1 and 1.0 $\mu\text{g}/\text{mL}$) and ATRA (10 μM) for 24 h in serum-free medium, and the expression of LRP2 mRNA was evaluated by realtime RT-PCR. The expression of LRP2 was not significantly changed by ATRA (Fig. 1B), but it was significantly decreased by LPS in a dose-dependent manner (Fig. 1A).

Uptake of A β and Insulin was Decreased by LPS Stimulation in Cultured Astrocytes

We examined the uptake of A β and insulin in cultured astrocytes to assess the effect of LRP2 expression changed by LPS. After the stimulation by 1.0 $\mu\text{g}/\text{mL}$ LPS for 24 h, the cells were incubated with 0.1 μM A β for 6 h and the uptake of A β was assessed by immunostaining. The ratio of A β and GFAP double-positive cells per GFAP-positive cells was decreased by LPS stimulation (Fig. 2A). Similarly, after the stimulation by 1.0 $\mu\text{g}/\text{mL}$ LPS for 24 h, the cells were incubated with 50 nM FITC-conjugated insulin for 30 min and the uptake of insulin was assessed under a fluorescent microscope. The ratio of FITC-conjugated insulin-positive

cells per total cells was decreased by LPS stimulation (Fig. 2B).

Changes of LRP2 mRNA Expression in BV-2 Cells

Next, we assessed in BV-2 cells the changes of LRP2 expression with various stimulation. The cells were incubated with LPS (0.001–1.0 $\mu\text{g}/\text{mL}$) and ATRA (1.0 and 10 μM) for 24 h in serum-free medium, and the expression of LRP2 mRNA was evaluated by realtime RT-PCR. The expression of LRP2 was significantly increased by LPS at 0.1 and 1.0 $\mu\text{g}/\text{mL}$ (Fig. 3A), and it was also significantly increased by 1.0 and 10 μM ATRA dose-dependently (Fig. 3B).

Uptake of A β and Insulin was Increased by LPS and ATRA Stimulation in BV-2 Cells

As described above, the expression of LRP2 mRNA was increased by LPS and ATRA treatments. Then, we examined the endocytosis activity of microbeads and the uptake of A β and insulin in BV-2 cells. After the cells were treated with 0.1 $\mu\text{g}/\text{mL}$ LPS or 1.0 μM ATRA for 24 h, the endocytosis of fluorescent microbeads was assessed. The endocytosis activity was significantly increased by LPS and ATRA (Fig. 4A). Similarly, after the stimulation by LPS or ATRA for 24 h, the cells were incubated with 0.1 μM A β for 6 h and the uptake of A β was assessed by immunostaining. The cellular fluorescence of A β -positive cells was increased by

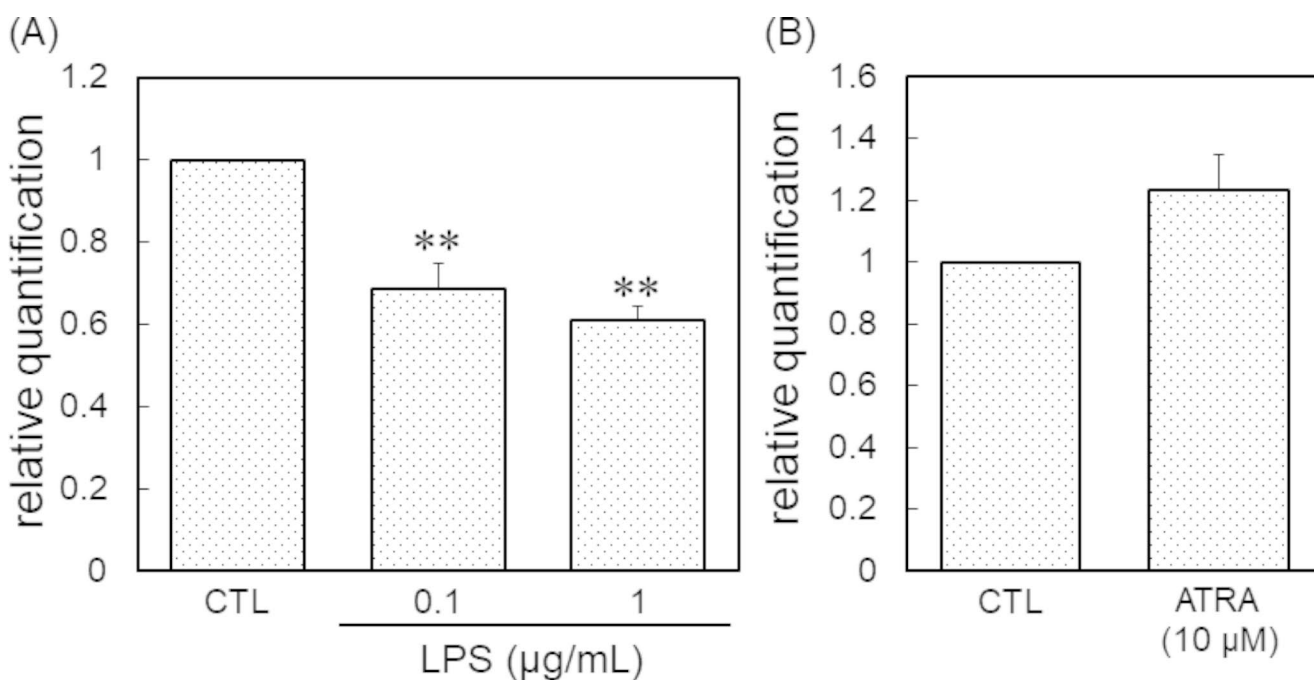


Fig. 1 Expression of LRP2 mRNA in cultured astrocytes treated with LPS or ATRA. Cultured astrocytes were treated with 0.1, 1.0 $\mu\text{g}/\text{mL}$ LPS (A) and 10 μM ATRA (B) for 24 h. The expression of mRNAs of LRP2 and 18S rRNA were assessed by realtime RT-PCR procedure.

Data are mean \pm S.D. of repeated 3–10 experiments. ** $p < 0.01$, significantly different from control (CTL) by Dunnet multiple comparison procedure

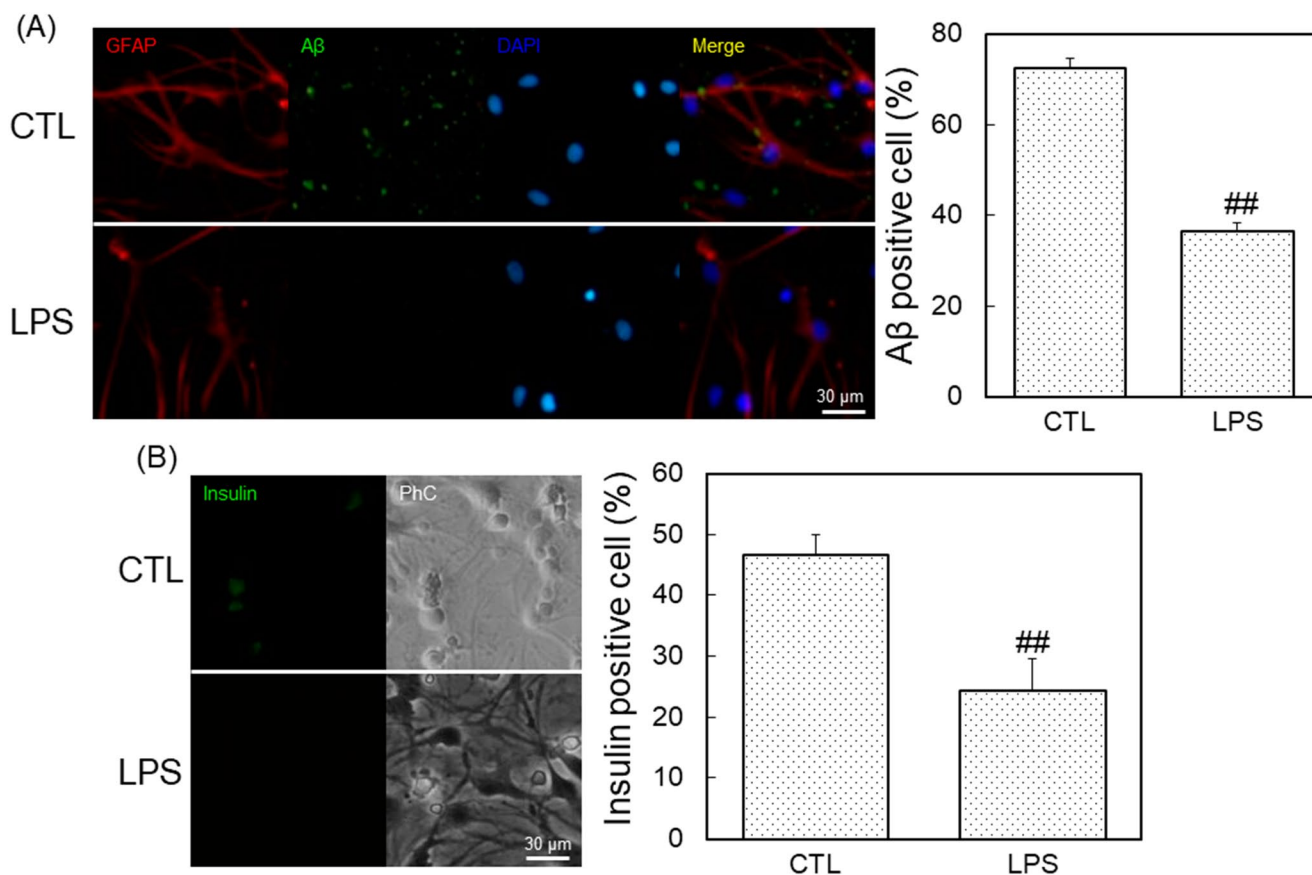


Fig. 2 Uptake of A β and insulin in cultured astrocytes treated with LPS. Cultured astrocytes were treated with 1.0 $\mu\text{g}/\text{mL}$ LPS for 24 h. (A) The uptake of A β by cultured astrocytes for 6 h was evaluated by immunostaining. The numbers of A β -positive cells and GFAP-positive astrocytes were counted ($n = 25\text{--}289$). The graph shows the ratio of A β and GFAP double-positive cells per GFAP-positive cells. The photograph shows representative fluorescent images at each same site. (B)

LPS and ATRA (Fig. 4B). Moreover, after stimulation, the cells were incubated with 50 nM FITC-conjugated insulin for 30 min and the uptake of insulin was also assessed under a fluorescent microscope. The ratio of FITC-conjugated insulin-positive cells per total cells was increased by LPS and ATRA stimulation (Fig. 4C).

LRP2 was Associated with Endocytosis Activity and Uptake of A β and Insulin in BV-2 Cells

To evaluate whether LRP2 might play roles in endocytosis and uptake of A β and insulin we studied using LRP2 knockdown in BV-2 cells. After LRP2 siRNA and negative control (CTL) siRNA were transfected in BV-2 cells for 24 h, the cells were treated with 0.1 $\mu\text{g}/\text{mL}$ LPS or 1.0 μM ATRA for 24 h, and the endocytosis of fluorescent microbeads and the uptake of A β and insulin were assessed. To confirm the reduction of LRP2 by siRNA, the expression of LRP2 mRNA was evaluated by realtime RT-PCR.

The uptake of insulin by cultured astrocytes for 30 min was evaluated. The numbers of FITC-conjugated insulin-positive cells and total cells were counted ($n = 81\text{--}748$). The graph shows the ratio of FITC-positive cells per total cells. The photograph shows representative phase contrast (PhC) and fluorescent images at each same site. Scale bar = 30 μm . Data are mean \pm S.D. of repeated three experiments: ### $p < 0.01$, significantly different from control (CTL) by Student's t test

Figure 5A showed in control group that LRP2 siRNA decreased LRP2 mRNA to about 60% compared with CTL siRNA (Fig. 5A). The endocytosis activity was significantly increased by LPS and ATRA in BV-2 cells transfected with control siRNA, while the increased endocytosis activity was completely suppressed in BV-2 cells transfected with LRP2 siRNA (Fig. 5B). The uptake of A β and insulin was also significantly increased by LPS and ATRA stimulation in BV-2 cells with control siRNA, and that was absolutely inhibited in BV-2 cells with LRP2 siRNA (Fig. 5C, D).

Discussion

In the present study, we demonstrated that LPS decreased LRP2 expression and the uptake of A β and insulin in cultured astrocytes. On the other hand, LPS increased LRP2 expression and the uptake of microbeads, A β and insulin in BV-2 microglial cell line. ATRA also increased LRP2

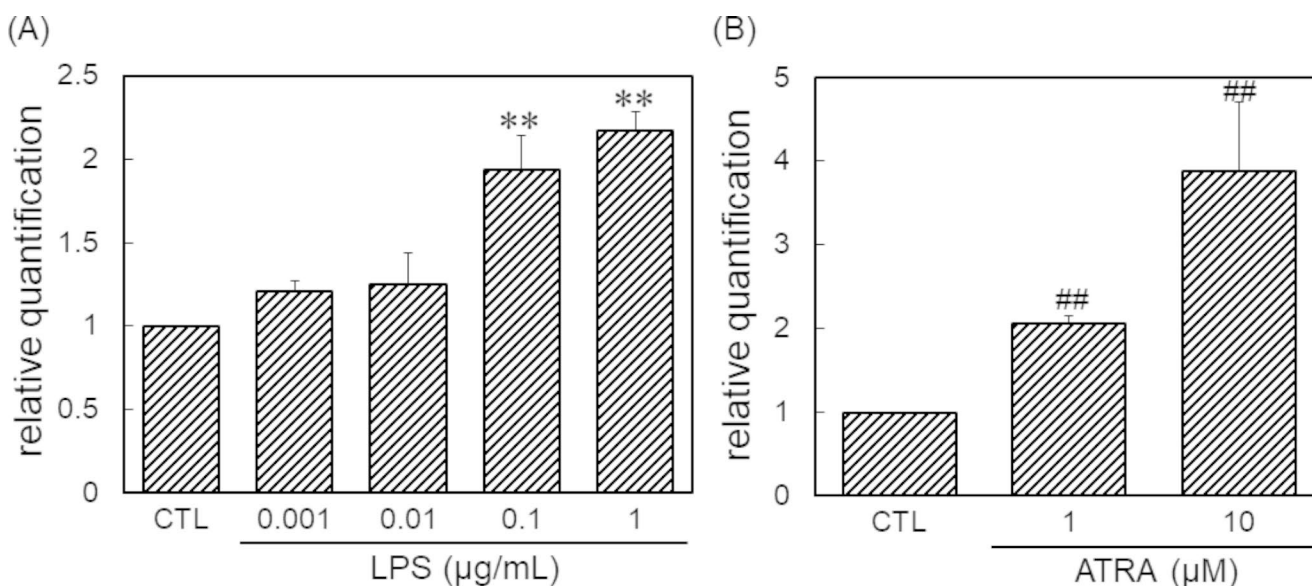


Fig. 3 Expression of LRP2 mRNA in BV-2 cells treated with LPS or ATRA. BV-2 cells were treated with various concentrations of LPS (A) and ATRA (B) for 24 h. The expression of mRNAs of LRP2 and 18S rRNA were assessed by realtime RT-PCR procedure. Data are

mean \pm S.D. of repeated 3–9 experiments. ** $p < 0.01$, significantly different from control (CTL) by Dunnett multiple comparison procedure. ## $p < 0.01$, significantly different from control (CTL) by Student's t test

expression and the uptake activity in BV-2 cells. LPS- and ATRA-increased the uptake activity in BV-2 cells was reduced by knockdown of LRP2. These results suggest that LRP2 expressed in glial cells might be involved in the uptake of microbeads, A β and insulin.

In the present study, we found that LRP2 mRNA expressions in cultured astrocytes (Fig. 1) and BV-2 cells (Fig. 3). Also in cultured microglia prepared from neonatal rat whole brain, LRP2 mRNA expression was confirmed (data not shown); therefore, at least mRNA levels of LRP2 expression in astrocytes and microglia were clarified. Unfortunately, we could not detect protein levels of LRP2 expression in these cells by western blot and immunocytochemistry analysis using commercial LRP2 antibodies. It was reported in mouse cultured astrocytes that LRP2 expression could not be detected using semi-quantitative PCR and immunocytochemistry [17]. The expression of LRP2 in glial cells might be limited and further investigation for LRP2 protein expression was needed.

LPS decreased LRP2 mRNA expression in cultured astrocytes (Fig. 1) and LPS and ATRA increased LRP2 expression in BV-2 cells (Fig. 3). Although transcription factor binding sites that are important for LRP2 expression have been identified [42], little is known about regulation of LRP2 mRNA expression [1]. Increased LRP2 expression was observed in neurons associated with AD that accumulate ApoE [13]. It was reported that oxidative stress increased LRP2 expression in renal proximal tubular cells [43] and LPS stimulation increased intracellular reactive oxygen species (ROS) production in microglia [44]. Therefore,

oxidative stress might be involved in LPS-increased LRP2 expression in BV-2 cells. LPS stimulation has been commonly used to activate glial cells in vitro experiments [37] and it has been reported that LPS binds to toll-like receptor (TLR) and activates both microglia and astrocytes through nuclear factor-kappa B (NF- κ B) signaling pathway [45, 46]. However, LRP2 mRNA expression in cultured astrocytes was decreased and that in BV-2 cells was increased by LPS in the present study. The downstream signaling of LPS for regulation of LRP2 mRNA expression would be considered in our future study.

It was reported that LRP2 expression in vitro was stimulated by retinoic acid (RA), dibutyryl cyclic AMP, and vitamin D [47, 48]. ATRA increased LRP2 expression and induced differentiation in prostate and colon epithelial cells [49]. Consistent with these reports, ATRA increased LRP2 expression in BV-2 cells in the present study (Fig. 3). On the other hand, LRP2 expression in cultured astrocytes was not significantly changed by ATRA treatments (Fig. 1). RA, ATRA and 9-cis RA, are metabolites of vitamin A that can modify the proliferation and differentiation of a variety of cells [39]. ATRA and 9-cis RA bind to the RA receptors (RAR α , β , and γ isotypes), whereas 9-cis RA binds to the retinoid receptors (RXR α , β , and γ isotypes), to regulate the expressions of various target genes [40]. RAR was thought to bind to RA-response elements (RAREs) in response to ATRA and promote transcription through interaction with coactivator [40]. It was known that astrocytes and microglia recognized ATRA via RAR/RXR causing functional changes [50, 51]. It was also reported that astrocytes

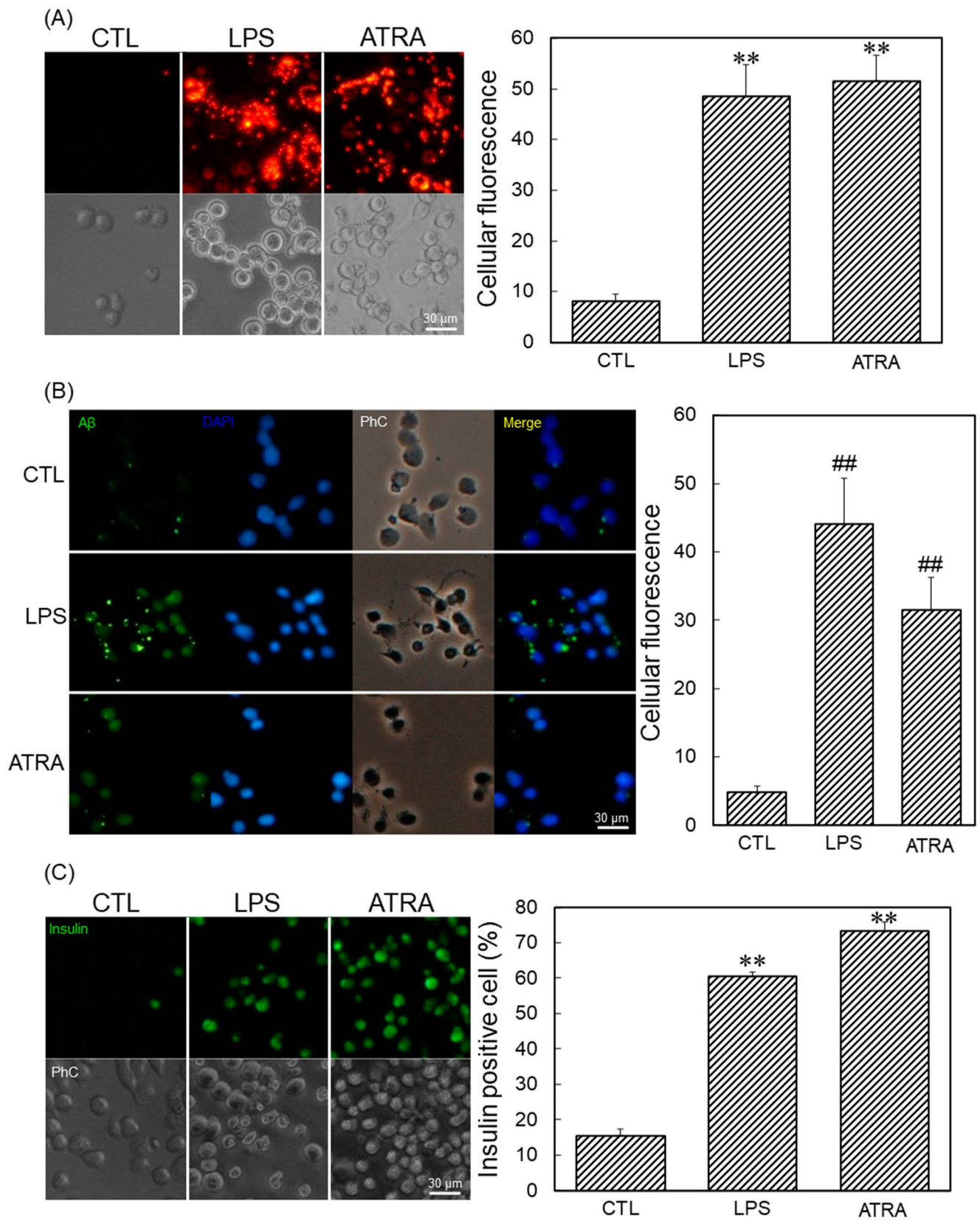


Fig. 4 Uptake of microbeads, A β and insulin in BV-2 cells treated with LPS or ATRA. BV-2 cells were treated with 0.1 μ g/mL LPS or 1.0 μ M ATRA for 24 h. (A) The uptake of fluorescent microbeads for 1 h was evaluated. The graph shows level of fluorescence per cell. The photograph shows representative phase contrast and fluorescent images at each same site. (B) The uptake of A β for 6 h was evaluated by immunostaining. The graph shows level of fluorescence per cell. The photograph shows representative phase contrast and fluorescent images at each same site. (C) The uptake of insulin for 30 min was evaluated. The numbers of FITC-conjugated insulin-positive cells and total cells were counted ($n = 179$ – 1433). The graph shows the ratio of FITC-positive cells per total cells. The photograph shows representative phase contrast and fluorescent images at each same site. Scale bar = 30 μ m. Data are mean \pm S.D. of repeated 3–5 experiments: ** $p < 0.01$, significantly different from control (CTL) by Tukey-Kramer multiple comparison procedure. ## $p < 0.01$, significantly different from control (CTL) by Student's t test

might be a regulated source of RA for the brain because the expression of retinal dehydrogenase, an enzyme for RA synthesis, in astrocytes changed according to the RA concentration [52]. Different changes of LRP2 expression in astrocytes and microglia by ATRA might be associated with ATRA concentration containing in the medium or using as the treatments in the present study. Further investigation was needed for the mechanisms of changes in LRP2 expression by ATRA.

ATRA had an anti-inflammatory function, decreasing the activation of astrocytes and microglia to inhibit the production of nitric oxide (NO) and inflammatory cytokines [53]. It was also known that ATRA had direct effects on neurogenesis to induce the differentiation of neuronal stem cell [50]. Therefore, ATRA might be a putative candidate drug for the treatment of patients with AD. The concentration of RA was reported as about 15 μ M in human serum, 5 μ M in rat serum, and 0.04–0.75 μ M in rat tissues [54]. Therefore, the concentration of ATRA used in the present study could occur in vivo. In our preliminary experiments, 1–10 μ M ATRA decreased LPS-induced NO production in cultured astrocytes and BV-2 cells consistent with previous research [41]. It was suggested that ATRA might inhibit LPS-induced NF- κ B activation [55]. However, both LPS and ATRA increased LRP2 expression in BV-2 cells in the present study (Fig. 3). The regulation of LRP2 mRNA might be associated with transcriptional factor other than NF- κ B and/or other mechanisms might be involved.

In the endocytosis assay of microbeads, A β and insulin, LPS decreased the uptake of A β and insulin in cultured astrocytes and LPS and ATRA increased the endocytosis activity in BV-2 cells correlated with LRP2 expression (Figs. 1, 2, 3 and 4). In addition, LRP2 knockdown by siRNA in BV-2 cells abolished the LPS- and ATRA-increased endocytosis of microbeads, A β and insulin (Fig. 5). Therefore, LRP2 might be involved in the endocytosis activity in microglia and related in astrocytes.

In the endocytosis of microbeads, no reports indicated direct interaction with LRP2. It has been reported that LRP2 binds to retinol binding protein and might be associated with the metabolism of vitamin A [56]. LRP2 was reported in macrophages to be a scavenger receptor endocytosing LDL [57]. Intracellular domain of LRP2 was cleaved and translocated into nuclear, and might interact with some transcription factors and regulate the expression of target genes [3]. Therefore, LPS- and ATRA-increased LRP2 expression in BV-2 cells might be involved in endocytosis through other related molecules. In our previous study, LPS and ATRA increased transglutaminase 2 expression and endocytosis of microbeads in BV-2 cells [in press]. It was suggested that LPS-increased transglutaminase 2 expression might be involved in endocytosis of microbeads [38].

Abnormal accumulation and aggregation of A β in brain is a characteristic pathology in AD, and it is reported in sporadic AD that A β production rate is not different from healthy brain while A β clearance is impaired [58]. Therefore, to elucidate the mechanism of A β uptake in astrocytes and microglia would be important for inhibition of A β accumulation. Several candidate molecules for A β uptake were reported; LRP, receptor of advanced glycation end product (RAGE), CD36 and CD47 [59]. LRP1 in astrocytes and microglia is known to mediate the uptake of A β [60, 61]. In the present study, the changes in LRP2 expressions after LPS and ATRA treatments had same direction of changes in the uptake of A β in both cultured astrocytes and BV-2 cells. LRP2 knockdown by siRNA in BV-2 cells abolished the LPS- and ATRA-increased endocytosis of A β . Therefore, not only LRP1 but also LRP2 might be associated with A β transport and clearance by microglia.

It was reported in kidney that LRP2 might play a significant role as a renal reabsorption receptor for the uptake of insulin [29]. However, none was known about the roles of LRP1 and LRP2 in insulin transport into brain. The results of present study suggested that LRP2 might be associated with endocytosis of insulin in astrocytes and microglia, similar to A β uptake. It has been reported that LRP1 in neurons interacts with insulin receptor and is involved in glucose transport [62]. The concentration of insulin in human cerebrospinal fluid was about 1 pM [63]. Increase of insulin levels augmented insulin resistance [64] and insulin resistance might be associated with the pathogenesis of AD [65]. Endocytosis of insulin via LRP2 in microglia might control the extracellular insulin levels in brain.

In conclusions, LRP2 expression in astrocytes and microglia might be related to endocytosis activity. Interestingly, activation by LPS caused opposite effects on LRP2 expression and endocytosis activities between astrocytes and microglia. In addition, both LPS, an activator, and ATRA, an inhibitor, of inflammatory responses of glial

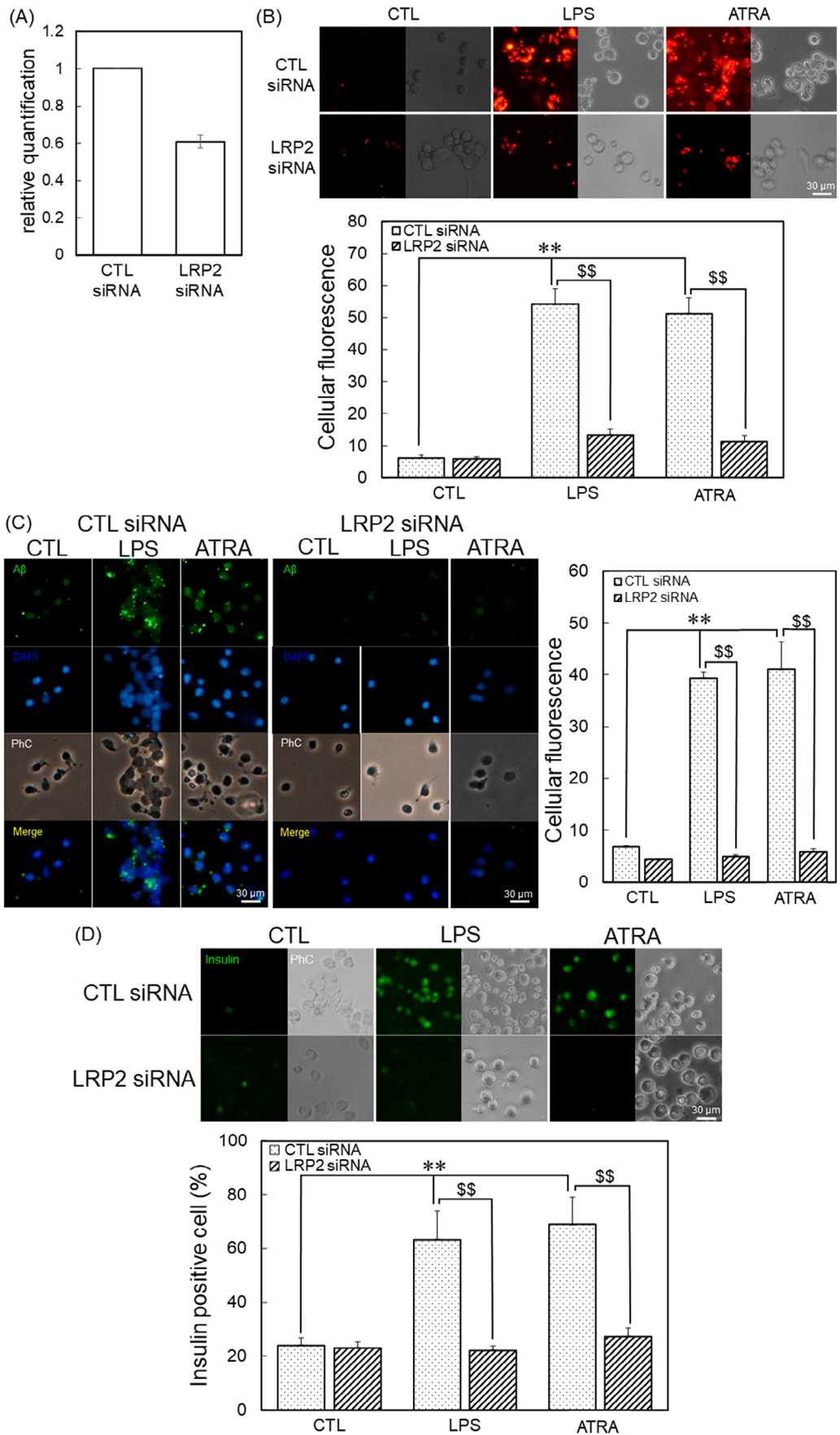


Fig. 5 Uptake of microbeads, A β and insulin in BV-2 cells transfected with LRP2 siRNA. BV-2 cells were transfected with negative control (CTL) and LRP2 siRNA for 24 h and treated with 0.1 μ g/mL LPS or 1.0 μ M ATRA for 24 h. (A) To confirm the knockdown of LRP2, the expression of mRNAs of LRP2 and 18S rRNA were assessed by real-time RT-PCR procedure. Data are mean \pm S.D. of repeated five samples. (B) The uptake of fluorescent microbeads for 1 h was evaluated. The graph shows level of fluorescence per cell. The photograph shows representative phase contrast and fluorescent images at each same site. (C) The uptake of A β for 6 h was evaluated by immunostaining. The graph shows level of fluorescence per cell. The photograph shows representative phase contrast and fluorescent images at each same site. (D) The uptake of insulin for 30 min was evaluated. The numbers of FITC-conjugated insulin-positive cells and total cells were counted (n = 105–1507). The graph shows the ratio of FITC-positive cells per total cells. The photograph shows representative phase contrast and fluorescent images at each same site. Scale bar = 30 μ m. Data are mean \pm S.D. of repeated 3–4 experiments: ** p < 0.01, significantly different from control (CTL) and ^{ss} p < 0.01, significantly different from CTL siRNA by Tukey-Kramer multiple comparison procedure

cells increased LRP2 expression and endocytosis in BV-2 cells. LRP2 expression and its regulations in glial functions would be complicated. LRP2 expression might be associated with AD pathology through the regulation of A β and insulin levels in brain and glial function; however, further investigation was needed to enclose the roles of LRP2 in AD pathology.

Acknowledgements This work was supported in part by JSPS KAKENHI Grant Number JP17K15390, JP19K06441 to K.T.

Author Contributions K. T.-K. wrote the main manuscript text and manage the study. K. M. performed the experiments and prepared the figures. Y. N and M. M. advised the study. All authors reviewed the manuscript.

Funding None.

Data Availability All data generated or analyzed during this study are included in this published article.

Declarations

Conflict of Interest The authors declare no conflict of interest, financial or otherwise.

References

- Christensen EI, Birn H (2002) Megalin and cubilin: multifunctional endocytic receptors. *Nat Rev Mol Cell Biol* 3(4):256–266. <https://doi.org/10.1038/nrm778>
- Beenken A, Gabriele Cerutti G, Brasch J, Guo Y, Sheng Z, Erdjument-Bromage H, Aziz Z, Robbins-Juarez SY, Chavez EY, Ahlsen G, Katsamba PS, Neubert TA, Fitzpatrick AWP, Barasch J, Shapiro L (2023) Structures of LRP2 reveal a molecular machine for endocytosis. *Cell* 186(4):821–836. <https://doi.org/10.1016/j.cell.2023.01.016>
- Spuch C, Ortolano S, Navarro C (2012) LRP-1 and LRP-2 receptors function in the membrane neuron. Trafficking mechanisms and proteolytic processing in Alzheimer's disease. *Front Physiol* 3:Article number 269. <https://doi.org/10.3389/fphys.2012.00269>
- Herz J, Bock HH (2002) Lipoprotein receptors in the nervous system. *Ann Rev Biochem* 71:405–434. <https://doi.org/10.1146/annurev.biochem.71.110601.135342>
- Bruno E, Quattrocchi G, Nicoletti A, Le Pira F, Maci T, Mottile G, Andreoli V, Quattrone A, Zappia M (2010) Lack of interaction between LRP1 and A2M polymorphisms for the risk of Alzheimer's disease. *Neurosci Lett* 482:112–116. <https://doi.org/10.1016/j.neulet.2010.07.012>
- Boucher P, Herz J (2011) Signaling through LRP1, protection from atherosclerosis and beyond. *Biochem Pharmacol* 81:1–5. <https://doi.org/10.1016/j.bcp.2010.09.018>
- Andersen OM, Willnow TE (2006) Lipoprotein receptors in Alzheimer's disease. *Trends Neurosci* 29:687–694. <https://doi.org/10.1016/j.tins.2006.09.002>
- Rebeck GW, Harr SD, Strickland DK, Hyman BT (1995) Multiple, diverse senile plaque-associated proteins are ligands of an apolipoprotein E receptor, the alpha 2-macroglobulin receptor/low-density-lipoprotein receptor related protein. *Ann Neurol* 37:211–217. <https://doi.org/10.1002/ana.410370212>
- Marzolo MP, von Bernhardt R, Bu G, Inestrosa NC (2000) Expression of α 2-macroglobulin receptor/low density lipoprotein receptor-related protein (LRP) in rat microglial cells. *J Neurosci Res* 60(3):401–411 doi: 10.1002/(SICI)1097-4547(20000501)60:3<401::AID-JNR15>3.0.CO;2-L
- Mauch DH, Nägler K, Schumacher S, Göritz C, Müller EC, Otto A, Pfrieger FW (2001) CNS synaptogenesis promoted by glia-derived cholesterol. *Science* 294(5545):1354–1357. <https://doi.org/10.1126/science.294.5545.1354>
- Pietrzik CU, Busse T, Merriam DE, Weggen S, Koo EH (2002) The cytoplasmic domain of the LDL receptor-related protein regulates multiple steps in APP processing. *EMBO J* 21(21):5691–5700. <https://doi.org/10.1093/emboj/cdf568>
- Kuhbandner K, Herz J, Pohlkamp T (2021) Chap. 18 - lipoprotein receptors. *Biochemistry of lipids, lipoproteins and membranes*. (Seventh Edition) 583–622. <https://doi.org/10.1016/B978-0-12-824048-9.00018-3>
- LaFerla FM, Troncoso JC, Strickland DK, Kawas CH, Jay G (1997) Neuronal cell death in Alzheimer's disease correlates with apoE uptake and intracellular A β stabilization. *J Clin Invest* 100(2):310–320. <https://doi.org/10.1172/JCI119536>
- Bento-Abreu A, Velasco A, Polo-Hernández E, Pérez-Reyes PL, Tabernero A, Medina JM (2008) Megalin is a receptor for albumin in astrocytes and is required for the synthesis of the neurotrophic factor oleic acid. *J Neurochem* 106(3):1149–1159. <https://doi.org/10.1111/j.1471-4159.2008.05462.x>
- Bento-Abreu A, Velasco A, Polo-Hernández E, Lillo C, Kozyraki R, Tabernero A, Medina JM (2009) Albumin endocytosis via megalin in astrocytes is caveola- and dab-1 dependent and is required for the synthesis of the neurotrophic factor oleic acid. *J Neurochem* 111(1):49–60. <https://doi.org/10.1111/j.1471-4159.2009.06304.x>
- Mulder SD, Veerhuis R, Blankenstein MA, Nielsen HM (2012) The effect of amyloid associated proteins on the expression of genes involved in amyloid- β clearance by adult human astrocytes. *Exp Neurol* 233(1):373–379. <https://doi.org/10.1016/j.expneurol.2011.11.001>
- Alvira-Botero X, Pérez-Gonzalez R, Spuch C, Vargas T, Antequera D, Garzón M, Bermejo-Pareja F, Carro E (2010) Megalin interacts with APP and the intracellular adapter protein FE65 in neurons. *Mol Cell Neurosci* 45(3):306–315. <https://doi.org/10.1016/j.mcn.2010.07.005>
- Page KJ, Hollister RD, Hyman BT (1998) Dissociation of apolipoprotein and apolipoprotein receptor response to lesion in the rat brain: an in situ hybridization study. *Neuroscience* 85(4):1161–1171. [https://doi.org/10.1016/s0306-4522\(97\)00661-1](https://doi.org/10.1016/s0306-4522(97)00661-1)

19. Kandimalla R, Thirumala V, Reddy PH (2016) Is Alzheimer's disease a type 3 diabetes? A critical appraisal. *Biochim Biophys Acta* 1863(5):1078–1089. <https://doi.org/10.1016/j.bbadis.2016.08.018>
20. Jin M, Shepardson N, Yang T, Chen G, Walsh D, Selkoe D (2011) Soluble amyloid beta protein dimers isolated from Alzheimer cortex directly induce tau hyperphosphorylation and neuritic degeneration. *Proc Natl Acad Sci USA* 108:5819–5824. <https://doi.org/10.1073/pnas.1017033108>
21. Zlokovic BV, Deane R, Sagare AP, Bell RD, Winkler EA (2010) Low-density lipoprotein receptor-related protein-1: a serial clearance homeostatic mechanism controlling Alzheimer's amyloid β peptide elimination from the brain. *J Neurochem* 115(5):1077–1089. <https://doi.org/10.1111/j.1471-4159.2010.07002.x>
22. Zlokovic BV (2004) Clearing amyloid through the blood-brain barrier. *J Neurochem* 89(4):807–811. <https://doi.org/10.1111/j.1471-4159.2004.02385.x>
23. Nuutinen T, Suuronen T, Kauppinen A, Salminen A (2009) Clusterin: a forgotten player in Alzheimer's disease. *Brain Res Rev* 61(2):89–104. <https://doi.org/10.1016/j.brainresrev.2009.05.007>
24. Liu RX, Huang C, Bennett DA, Li H, Wang R (2016) The characteristics of astrocyte on A β clearance altered in Alzheimer's disease were reversed by anti-inflammatory agent (+)-2-(1-hydroxyl-4-oxocyclohexyl) ethyl caffeate. *Am J Transl Res* 8(10):4082–4094. PMID: 27829994, PMCID: PMC5095303, Free PMC article
25. Nieuoullon A (2011) Neurodegenerative diseases and neuroprotection: current views and prospects. *J Appl Biomed* 9:173–183. <https://doi.org/10.2478/v10136-011-0013-4>
26. Jaeger LB, Dohgu S, Hwang MC, Farr SA, Murphy MP, Fleeagal-Demotta MA, Lynch JL, Robinson SM, Niehoff ML, Johnson SN, Kumar VB, Banks WA (2009) Testing the neurovascular hypothesis of Alzheimer's disease: LRP-1 antisense reduces blood-brain barrier clearance, increases brain levels of amyloid- β protein, and impairs cognition. *J Alzheimer's Dis* 17(3):553–570. <https://doi.org/10.3233/JAD-2009-1074>
27. Dietrich M, Antequera D, Pascual C, Castro N, Bolos M, Carro E (2014) Alzheimer's disease-like impaired cognition in endothelial-specific megalin-null mice. *J Alzheimer's Dis* 39(4):711–717. <https://doi.org/10.3233/JAD-131604>
28. Pascale CL, Miller MC, Chiu C, Boylan M, Caralopoulos IN, Gonzalez L, Johanson CE, Silverberg GD (2011) Amyloid-beta transporter expression at the blood-CSF barrier is age-dependent. *Fluids Barriers CNS* 8(1): Article number 21. <https://doi.org/10.1186/2045-8118-8-21>
29. Orlando RA, Rader K, Authier F, Yamazaki H, Posner BI, Bergeron JJM, Farquhar MG (1998) Megalin is an endocytic receptor for insulin. *J Am Soc Nephrol* 9(10):1759–1766. <https://doi.org/10.1681/ASN.V9101759>
30. Nemoto T, Toyoshima-Aoyama F, Yanagita T, Maruta T, Fujita H, Koshida T, Yonaha T, Wada A, Sawaguchi A, Murakami M (2013) New insights concerning insulin synthesis and its secretion in rat hippocampus and cerebral cortex: amyloid β_{1-42} -induced reduction of proinsulin level via glycogen synthase kinase-3 β . *Cell Signal* 26:253–259. <https://doi.org/10.1016/j.cellsig.2013.11.017>
31. Zhao WQ, Fernanda G, Sara F, Chen H, Lambert MP, Quon MJ, Krafft GA, Klein WL (2008) Amyloid beta oligomers induce impairment of neuronal insulin receptors. *FASEB J* 22:246–260. <https://doi.org/10.1096/fj.06-7703com>
32. Tokutake T, Kasuga K, Yajima R, Sekine Y, Tezuka T, Nishizawa M, Ikeuchi T (2012) Hyperphosphorylation of tau induced by naturally secreted amyloid- β at nanomolar concentrations is modulated by insulin-dependent Akt-GSK3 β signaling pathway. *J Biol Chem* 287(42):35222–35233. <https://doi.org/10.1074/jbc.M112.348300>
33. Craft S, Peskind E, Schwartz W, Gerard D, Raskind M, Porte D Jr (1998) Cerebrospinal fluid and plasma insulin levels in Alzheimer's disease. *Neurology* 50(1):164–168. <https://doi.org/10.1212/wnl.50.1.164>
34. Takano K, Koarashi K, Kawabe K, Itakura M, Nakajima H, Moriyama M, Nakamura Y (2018) Insulin expression in cultured astrocytes and the decrease by amyloid β . *Neurochem Int* 119:171–177. <https://doi.org/10.1016/j.neuint.2017.10.017>
35. Banks WA (2004) The source of cerebral insulin. *Eur J Pharmacol* 490(1–3):5–12. <https://doi.org/10.1016/j.ejphar.2004.02.040>
36. de la Monte SM (2017) Insulin resistance and neurodegeneration: Progress towards the development of new therapeutics for Alzheimer's disease. *Drugs* 77(1):47–65. <https://doi.org/10.1007/s40265-016-0674-0>
37. Takano K, Shiraiwa M, Moriyama M, Nakamura Y (2010) Transglutaminase 2 expression induced by lipopolysaccharide stimulation together with NO synthase induction in cultured astrocytes. *Neurochem Int* 57(7):812–818. <https://doi.org/10.1016/j.neuint.2010.08.019>
38. Kawabe K, Takano K, Moriyama M, Nakamura Y (2015) Lipopolysaccharide-stimulated transglutaminase 2 expression enhances endocytosis activity in the mouse microglial cell line BV-2. *Neuroimmunomodulation* 22:243–249. <https://doi.org/10.1159/000365484>
39. Kaur C, Sivakumar V, Dheen ST, Ling EA (2006) Insulin-like growth factor I and II expression and modulation in amoeboid microglial cells by lipopolysaccharide and retinoic acid. *Neuroscience* 138:1233–1244. <https://doi.org/10.1016/j.neuroscience.2005.12.025>
40. Lo HM, Wang SW, Chen CL, Wu PH, Wu WB (2014) Effects of all-trans retinoic acid, retinol, and β -carotene on murine macrophage activity. *Food Funct* 5:140–148. <https://doi.org/10.1039/c3fo60309a>
41. Takamura R, Watamura N, Nikkuni M, Ohshima T (2017) All-trans retinoic acid improved impaired proliferation of neural stem cells and suppressed microglial activation in the hippocampus in an Alzheimer's mouse model. *J Neurosci Res* 95:897–906. <https://doi.org/10.1002/jnr.23843>
42. Zhao J, Oleinikov AV, Oleinikova I, Makker SP (2001) Functional characterization of rat gp600/megalin promoter: combination of proximal Sp1 site and JCV repeat is important in rat gp600/megalin promoter activation. *Gene* 265:123–131. [https://doi.org/10.1016/s0378-1119\(01\)00351-1](https://doi.org/10.1016/s0378-1119(01)00351-1)
43. Kurosaki Y, Imoto A, Yokoba M, Takenaka T, Katagiri M, Ichikawa T, Ishii T (2017) Oxidative stress increases megalin expression through PI3K/Akt signaling in renal proximal tubular cells. *Nephrol Dialysis Transplant* 32(3):iii613. <https://doi.org/10.1152/ajprenal.00108.2017>
44. Qin L, Liu Y, Hong J-S, Crews FT (2013) NADPH oxidase and aging drive microglial activation, oxidative stress, and dopaminergic neurodegeneration following systemic LPS administration. *Glia* 61(6):855–868. <https://doi.org/10.1002/glia.22479>
45. Lee SY, Son DJ, Lee YK, Lee JW, Lee HJ, Yun YW, Ha TY, Hong JT (2006) Inhibitory effect of sesaminol glucosides on lipopolysaccharide-induced NF- κ B activation and target gene expression in cultured rat astrocytes. *Neurosci Res* 56:204–212. <https://doi.org/10.1016/j.neures.2006.06.005>
46. Zhao J, Bi W, Xiao S, Lan X, Cheng X, Zhang J, Lu D, Wei W, Wang Y, Li H, Fu Y, Zhu L (2019) Neuroinflammation induced by lipopolysaccharide causes cognitive impairment in mice. *Sci Rep* 9(1):5790. <https://doi.org/10.1038/s41598-019-42286-8>
47. Stefansson S, Chappell DA, Argraves KM, Strickland DK, Argraves WS (1995) Glycoprotein 330/low density lipoprotein receptor-related protein-2 mediates endocytosis of low density lipoproteins via interaction with apolipoprotein B100.

- J Biol Chem 270(33):19417–19421. <https://doi.org/10.1074/jbc.270.33.19417>
48. Liu W, Yu WR, Carling T, Juhlin C, Rastad J, Ridefelt P, Akerström G, Hellman P (1998) Regulation of gp330/megalin expression by vitamins A and D. *Eur J Clin Invest* 28(2):100–107. <https://doi.org/10.1046/j.1365-2362.1998.00253.x>
 49. Ternes SB, Rowling MJ (2013) Vitamin D transport proteins megalin and disabled-2 are expressed in prostate and colon epithelial cells and are induced and activated by all-trans-retinoic acid. *Nutr Cancer* 65(6):900–907. <https://doi.org/10.1080/01635581.2013.805422>
 50. Hellmann-Regen J, Kronenberg G, Uhlemann R, Freyer D, Endres M, Gertz K (2013) Accelerated degradation of retinoic acid by activated microglia. *J Neuroimmunol* 256(1–2):1–6. <https://doi.org/10.1016/j.jneuroim.2012.11.005>
 51. Zhao J, Fu Y, Liu CC, Shinohara M, Nielsen HM, Dong Q, Kanekiyo T, Bu G (2014) Retinoic acid isomers facilitate apolipoprotein E production and lipidation in astrocytes through the retinoid X receptor/retinoic acid receptor pathway. *J Biol Chem* 289(16):11282–11292. <https://doi.org/10.1074/jbc.M113.526095>
 52. Shearer KD, Fragoso YD, Clagett-Dame M, McCaffery PJ (2012) Astrocytes as a regulated source of retinoic acid for the brain. *Glia* 60(12):1964–1976. <https://doi.org/10.1002/glia.22412>
 53. Fukasawa H, Nakagomi M, Yamagata N, Katsuki H, Kawahara K, Kitaoka K, Miki T, Shudo K (2012) Tamibarotene: a candidate retinoid drug for Alzheimer's disease. *Biol Pharm Bull* 35(8):1206–1212. <https://doi.org/10.1248/bpb.b12-00314>
 54. Napoli JL (1986) Quantification of physiological levels of retinoic acid. *Methods Enzymol* 123(1):112–124. [https://doi.org/10.1016/s0076-6879\(86\)23015-3](https://doi.org/10.1016/s0076-6879(86)23015-3)
 55. Wang R, Chen S, Liu Y, Diao S, Xue Y, You X, Park EA, Liao FF (2015) All-trans-retinoic acid reduces BACE1 expression under inflammatory conditions via modulation of nuclear factor κ B (NF κ B) signaling. *J Biol Chem* 290:22532–22542. <https://doi.org/10.1074/jbc.M115.662908>
 56. Christensen EI, Nielsen R (2007) Role of megalin and cubilin in renal physiology and pathophysiology. *Rev Physiol Biochem Pharmacol* 158:1–22. https://doi.org/10.1007/112_0604
 57. Herz J, Strickland DK (2001) LRP: a multifunctional scavenger and signaling receptor. *J Clin Invest* 108(6):779–784. <https://doi.org/10.1172/JCI13992>
 58. Mawuenyega KG, Sigurdson W, Ovod V, Munsell L, Kasten T, Morris JC, Yarasheski KE, Bateman RJ (2010) Decreased clearance of CNS amyloid- β in Alzheimer's disease. *Science* 330(6012):1774. <https://doi.org/10.1126/science.1197623>
 59. Jones RS, Minogue AM, Connor TJ, Lynch MA (2013) Amyloid- β -induced astrocytic phagocytosis is mediated by CD36, CD47 and RAGE. *J Neuroimmune Pharmacol* 8(1):301–311. <https://doi.org/10.1007/s11481-012-9427-3>
 60. Laporte V, Lombard Y, Levy-Benezra R, Tranchant C, Poindron P, Warter JM (2004) Uptake of A β 1-40- and A β 1-42-coated yeast by microglial cells: a role for LRP. *J Leukoc Biol* 76(2):451–461. <https://doi.org/10.1189/jlb.1203620>
 61. Liu CC, Hu J, Zhao N, Wang J, Wang N, Cirrito JR, Kanekiyo T, Holtzman DM, Bu G (2017) Astrocytic LRP1 mediates brain A β clearance and impacts amyloid deposition. *J Neurosci* 37(15):4023–4031. <https://doi.org/10.1523/JNEUROSCI.3442-16.2017>
 62. Liu CC, Hu J, Tsai CW, Yue M, Melrose HL, Kanekiyo T, Bu G (2015) Neuronal LRP1 regulates glucose metabolism and insulin signaling in the brain. *J Neurosci* 35(14):5851–5859. <https://doi.org/10.1523/JNEUROSCI.5180-14.2015>
 63. Wallum BJ, Taborsky GJ, Porte D, Figlewicz DP, Jacobson L, Beard JC, Ward WK, Dorsa D (1987) Cerebrospinal fluid insulin levels increase during intravenous insulin infusions in man. *J Clin Endocrinol Metab* 64(1):190–194. <https://doi.org/10.1210/jcem-64-1-190>
 64. Wilcox G (2005) Insulin and insulin resistance. *Clin Biochem Rev* 26(2):19–39. PMID: 16278749, PMCID: PMC1204764, Free PMC article
 65. Watson GS, Craft S (2003) The role of insulin resistance in the pathogenesis of Alzheimer's disease: implications for treatment. *CNS Drugs* 17(1):27–45. <https://doi.org/10.2165/00023210-200317010-00003>

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Springer Nature or its licensor (e.g. a society or other partner) holds exclusive rights to this article under a publishing agreement with the author(s) or other rightsholder(s); author self-archiving of the accepted manuscript version of this article is solely governed by the terms of such publishing agreement and applicable law.