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The Expression Profiles of Lysophospholipid Receptors (LPLRs) in Different Endothelial Cells

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ABSTRACT: Sphingosine-1-phosphate (S1P) and lysophosphatidic acid (LPA) are two bioactive lysophospholipids (LPLs), stored primarily in platelets and released during platelet activation. Both LPLs are capable of regulating endothelial cell functions. The physiological functions of S1P and LPA are mediated by interacting with eight different G-protein coupled receptors: S1P1 through 5 and LPA1 through 3, which activate three different heterotrimeric GTP proteins－including Gi、Gq and G(12/13). The expression of LPL receptors in endothelial cells would affect the responses of S1P and LPA to these cells. There is no previous report discussing the expression profiles of LPL receptors in different endothelial cells from various species. In this study, we aim to investigate the expression profiles of S1P and LPA receptors in different endothelial cells isolated from human, rat, mouse and bovine origin. We used RT-PCR to determine LPLs receptors expression profiles in different endothelial cells. Our results indicated that endothelial cells from various species express different LPL receptors. Endothelial cells isolated from the same source of different species also had different LPLs receptors expression profiles. Therefore, different endothelial cells should respond to LPLs in different manners.

KEY WORDS: LPA, S1P, Endothelial cell, Lysophospholipid receptors (LPLRs).

INTRODUCTION

Endothelial cells are a popular model nowadays in investigating angiogenesis. They are the inner most layer of the blood vessel, and play critical roles in the control of vascular functions (Michiels, 2003). Endothelial cells participate in multiple aspects of the vascular homeostasis and regulating physiological and pathological processes like thrombosis, inflammation, and remodeling of vascular wall (Biedermann, 2001). They are also gatekeepers in leukocyte recruitment to inflammatory foci and lymphocyte homing to secondary lymphoid organs (Lampugnani et al., 1993). The development of functional vascular network requires a remarkable coordination between cells and growth factors, such as VEGF and angiopoietins (Risau et al., 1998; Iizasa et al., 2002; Wright et al., 2002).

LPLs are generated from precursors stored in membranes and secreted by platelets, macrophages, epithelial cells and some cancer cells in amounts sufficient to establish micromolar concentrations in serum and in other extracellular fluids during tissue reactions (Bishop and Bell, 1988; le Balle, 1999). In plasma, concentrations of S1P and LPA are approximately 200 nM (Yatomi et al., 2001) and 2 nM (Pages et al., 2001). They are stored primarily in platelets, presumably due to the presence of sphingosine kinase and the absence of sphingosine lyase (Siess et al., 1999; Kimura et al., 2001; Yatomi et al., 2001). Recent evidences suggested that autotoxin, a previously identified exo-enzyme, might be responsible for the generation of these two phospholipids (Umezu-Goto et al., 2002; Clair et al., 2003; Ferry et al., 2003).

S1P and LPA are capable of regulating multiple aspects of angiogenesis and vessel maturation *in vitro* and *in vivo*, including endothelial cell chemotaxis, differentiation, proliferation, migration, survival and capillary morphogenesis (Tamama et al., 2002). S1P and LPA may act as autocrine and paracrine mediators on cells of developing vascular bed by interacting with members of G-protein coupled receptors: S1P1 through 5 and LPA1 through 3, which could activate more than three different heterotrimeric GTP protein－Gi、Gq and G(12/13), mediate different physiological functions (Spiegel, 1996; Wang et al., 1999; Xie et al., 2002). The specific expression of these receptors among different

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species, coupled with their diverse cellular functions, has made lysophospholipid receptors an important focus of signal transduction research (Toman et al., 2002).

The endothelia of different tissues are influenced by their own unique and specific environmental stimuli or original genetic predisposition. These varieties cause endothelium to be a heterogeneous population (Ribatti et al., 2002). Endothelium differs not only in its phenotype, but also its surface protein expression, mRNA expression (Belloni et al., 1988) and responses to growth factors. For instance, the PECAM-1 expression is down-regulated in human umbilical vein endothelial cells (HUVECs) by TNF-α activation, but its expression is increased in human dermal microvascular ECs (HMVECs) (Ribatti et al., 2002). The endothelial cell growth factor (ECGF) had mitogenic effects in almost all endothelial cells tested regardless of tissue origin, but the stimulation effect of epidermal growth factor (EGF) was only observed on liver-derived hepatic sinusoidal endothelial cells (Belloni et al., 1992). The endothelial cells between large and small vessels and between arterial and venous vessels also express different factors (Wang et al., 1998; Helbling et al., 2000). Besides, the primary culture of isolated endothelial cells may alter their characteristics in original tissue (Borsum et al., 1982). Due to the various heterogeneities of endothelial cells, choosing consistent endothelial cells as a model system is critical to study endothelial cell physiology.

Different LPLs receptors expression in endothelial cells would affect the S1P and LPA functions. Therefore, to investigate the LPLs receptors expression on cells is relevant to understand the physiological effects of S1P and LPA. In this study, we aim to investigate the expression profiles of LPA and S1P receptors in different endothelial cells isolated from human, rat, mouse and bovine origins. Without antibodies against LPL receptors, RT-PCR is a useful method to investigate receptor expression profiles on endothelial cells. To assure our PCR results, all PCR reactions are performed with known positive controls. We also used β-actin or GAPDH primers to confirm the quality of cDNAs. Our results indicated that endothelial cells from various species express different LPL receptor profiles. Furthermore, endothelial cells isolated from the same source of different species also had different LPLs receptors expression profiles. In addition, PCR products of bovine LPLs receptors were sequenced and found highly conserved with known mRNA sequences from other species.

MATERIALS AND METHODS

Reagents

Sphingosine-1-Phosphate (S1P), lysophosphatidic acid (LPA) were purchased from Sigma (St. Louis, MO, USA.). Dulbecco's modified Eagle's medium (DMEM), Fetal bovine serum (FBS) were obtained from Hyclone Laboratories (Logan, UT). Penicillin-Streptomycin and Trypsin-EDTA were purchased from Invitrogen Corporation (Grand island, N.Y., USA.).

Primary culture of rat aortic endothelial cells (RAECs)

One-month-old Wistar rats were purchased from National Taiwan University Hospital (Taipei, Taiwan) and anesthetized with standard protocol (Penal barbital, 1ml/Kg). Thoracic aorta was collected with sterile process. The connective tissues attached on the blood vessel were mechanically removed and erythrocytes were washed out with DMEM. The cleaned vessel was cross cut with blade and 2mm thick vessel rings were cultured on 35mm dishes. Rings were transferred onto a new dish every two days. The cells adhered on the 2-4 transferred dishes were cultured as rat aortic endothelial cells (RAECs) and characterized as endothelial cells for the presence of von Willebrand factor VIII by immunocytochemistry with FITC-labeled antibodies (Chemicon, Temecula, CA, USA.). They were routinely cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1.5 mM glutamine, 100 IU/mL penicillin, and 50 ng/mL streptomycin (complete media). Culture flasks were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Primary culture of human umbilical vein endothelial cells (HUVECs)

Human umbilical cords were kindly provided from Chun-shai and U-Shen Hospital (Taipei, Taiwan). The umbilical cord was washed with cold 1X PBS and then incubated with 0.1% collagenase IV (Sigma, St. Louis, MO, USA.) in 37°C incubator for 8 minutes. After the incubation, the umbilical vein EC were washed out with serum-free DMEM and cultured with 20% EGM in 80% DMEM with 4mM L-glutamine adjusted to 1.5 g/L sodium bicarbonate, 4.5 g/L glucose, 1.0 mM sodium pyruvate, 100 IU/ml penicillin, 50 ng/mL streptomycin and 10% FBS. Culture flasks were maintained at 37 °C in a humidified atmosphere of 5% CO_2.

Isolation and Routine Culture of bovine aortic endothelial cells（BAECs）

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BAEC were kindly provided by Dr. Shu Chien (Department of Bioengineering, UCSD, CA, USA.) and characterized as endothelial cells by the presence of von Willebrand factor and the uptake of 1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocya nine perchlorate (Dil)-labeled acetylated low density lipoprotein. They were routinely cultured in Dulbecco's modified Eagle's medium containing 10% fetal bovine serum, 1.5 mM glutamine, 100 IU/mL penicillin, and 50 ng/mL streptomycin. Culture flasks were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Culture of BCECs, MS1, BUVECs and HAECs

Proliferating bovine cornea endothelial cells (BCECs) and mouse pancreatic islet endothelial cells (MS-1) were purchased from Food Industry Research and Development Institute (Hsinchu, Taiwan). Human aortic endothelial cells (HAECs) were kindly provided by Dr. Yuh-Lien Chen (Institute of Anatomy and Cell Biology, National Taiwan University Hospital). Bovine umbilical vein endothelial cells (BUVECs) were kindly provided by Dr. Carmen Clapp (Neurobiology Center, National Autonomous University of Mexico, Queretaro, Mexico). BCECs, HAECs and BUVECs were cultured in Dulbecco's modified Eagle's medium (Hyclone, Logan, Utah) containing 10% (v/v) fetal bovine serum (Hyclone, Logan, Utah) and MS1 cells were cultured in Dulbecco's modified Eagle's medium containing 5% (v/v) fetal bovine serum. Culture flasks were maintained at 37°C in a humidified atmosphere of 5% CO_2.

Reverse Trancript (RT)-PCR

Endothelial cells were lysed in TRIzol (Invitrogen, Carlsbad, CA) and total RNA from the samples were extracted. One μg total RNA were mixed with 1μl dNTP (Viogene, Taiwan) and 1μl oligo dT (MDBio Inc., Taiwan) for 5 minutes at 65°C. Then, the RNA was reverse-transcribed for 60 min at 42°C using reverse transcriptase enzyme (NEB Inc., Beverly, MA). For PCR amplification, specific primer sets were designed based on each LPLs receptors sequences on NCBI (Table 1). -actin or GAPDH was used to confirm the quality of reverse-transcripted cDNA and previous PCR products or genomic DNA were used as internal controls.

PCR was carried out in a final volume of 50 μL containing 2 μL of cDNA, 1 μL of dNTP (10 mM), 5 μL of 10× PCR buffer (10 mM Tris-HCl, pH 9, 50 mM KCl and 0.1% Triton X-100, 15 mM $MgCl_2$), 2 μl of sense- and antisense-specific oligonucleotide primers (10 μM), and 1.25 units of *Taq* DNA polymerase (Geneaid, Taiwan). Conditions for the PCR reaction were: initial denaturation step at 94°C for 5 min, followed by 35 cycles consisting in 30 sec at 94°C, 45 sec at 52-62°C, 72°C for 1 min. After a final extension at 72°C for 10 min, PCR products were separated on 2% agarose gel, and amplified products were visualized with ethidium bromide and photographed. PCR products were sequenced and aligned with known species using ClustalW software.

RESULTS

The primer design for LPL receptors for four different species

The current published LPLs receptors mRNA sequences in human, rat, mouse and bovine species in NCBI are illustrate in Table 1. Both human and mouse LPLs receptors mRNA sequences are completely identified. On the other hand, mRNA sequences of S1P4 and LPA1 receptors in rat have not yet been published. Only the LPA1 receptor mRNA sequence is identified in bovine. Therefore, the primers sets for the four species were designed according to the existing mRNA sequences or on highly conserved regions by aligning known receptor mRNA sequence in other species. As shown in Table 2 and Table 3, human and mouse LPLs receptors primer sets were designed according to the existing mRNA sequences in NCBI. Rat S1P4 and LPA2 primer sets were designed from known human and mouse mRNA sequences (Table 4). Bovine S1P1-5 and LPA2-3 primer sets were also designed from the conserved regions of human, mouse and rat mRNA sequences of the receptors (Table 5). Each primer set has specific optimal annealing temperature for PCR reaction (Tables 2-5).

Endothelial cells from different species express different LPLs receptors

As illustrate in our results, the LPLs receptors expression on human umbilical vein endothelial cells (HUVECs), HUVEC hybridoma cell line (EAhy) and human aortic endothelial cells (HAECs) were different (Figs. 1-3 and Table 6). In agreement with previous publications (Wang et al., 1999; Lee et al., 2000; Paik et al., 2001), HUVECs expressed S1P1, 3, 4 and 5 as well as LPA1 and 3. EAhy cells expressed S1P1, 5 and LPA1, 3. HAECs expressed only S1P1 and LPA1, 3 (Figs. 1-3 and Table 6). S1P1 and LPA1 as well as LPA3 were expressed on all three human endothelial cells. None of these human endothelial cells express S1P2 and LPA2. In our results, we concluded that different human endothelial cells expressed different LPLs receptors. The different LPLs receptors expression profiles on various endothelial cells might affect the LPLs mediated endothelial physiological functions.

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Table 1. Accession numbers for LPL receptors on NCBI.

Species	Human	Rat	Mouse	Bovine
S1P1	XM_001400	NM_017301	NM_007901	--
S1P2	NM_004230	NM_017192	XM_134731	--
S1P3	NM_005226	AF184914	NM_010101	--
S1P4	NM_003775	--	NM_010102	--
S1P5	NM_030760	NM_021775	NM_053190	--
LPA1	NM_057159	NM_053936	NM_010336	NM_174047
LPA2	NM_004720	--	NM_020028	--
LPA3	NM_012152	NM_023969	NM_022983	--

-- = not identified

Table 2. Human LPLs receptors primer set.

Receptors	Primer sequences	Product size	Temp.
S1P1	5' ^{1273}GACTCTGCTGGCAAATTCAAGCGAC^{1297} 3' ^{1624}ACCCTTCCCAGTGCATTGTTCACAG^{1600}	352 b.p.	62°C
S1P2	5' ^{551}CTCTCTACGCCAAGCATTATGTGCT^{575} 3' ^{1062}TCAGACCACCGTGTTGCCCTC^{1042}	512 b.p.	62°C
S1P3	5' ^{819}CAAAATGAGGCCTTACGACGCCA^{841} 3' ^{1519}TCCCATTCTGAAGTGCTGCGTTC^{1497}	701 b.p.	61.2°C
S1P4	5' ^{605}AGCCTTCTGCCCCTCTACTC^{624} 3' ^{943}GTAGATGATGGGGTTGACCG^{924}	339 b.p.	62°C
S1P5	5' ^{1732}GGAGTAGTTCCCGAAGGACC^{1751} 3' ^{1967}TCTAGAATCCACGGGGTCTG^{1948}	236 b.p.	59.7°C
LPA1	5' ^{733}CGGAGACTGACTGTCAGCAC^{752} 3' ^{1130}GGTCCAGAACTATGCCGAGA^{1111}	397 b.p.	62°C
LPA2	5' ^{416}AGCTGCACAGCCGCCTGCCCCGT^{438} 3' ^{1303}TGCTGTGCCATGCCAGACCTTGTC^{1280}	888 b.p.	53°C
LPA3	5' ^{256}TTAGCTGCTGCCGATTTCTT^{275} 3' ^{647}ATGATGAGGAAGGCCATGAG^{628}	392 b.p.	62°C
β-actin	5' ^{341}TTCTACAATGAGCTGCGTGTGGC^{363} 3' ^{1154}CCTGCTTGCTGATCCACATCTGC^{1132}	814 b.p.	62°C

Table 3. Mouse LPLs receptors primer set.

Receptor	Primer sequence	Product size	Temp.
S1P1	5' ^{587}GTCCGGCATTACAACTACAC^{606} 3'^{1029}ATGAGGGAGATGACCCAGCA^{1010}	443 b.p.	60°C
S1P2	5' ^{82}ACCGAGCACAGCCAACAGTC^{101} 3' ^{451}GCCAGGTTGCCAAGGAACAG^{432}	370 b.p.	60°C
S1P3	5' ^{600}GCTGGCCGGCATAGCATA^{618} 3' ^{1110}GGATAAAAAGTGGGGACC^{1093}	511 b.p.	60°C
S1P4	5' ^{1200}CCAATGGGCAGAAGTCTCCA^{1020} 3' ^{1660}CTAGGTGCTGCGGACGCT^{1643}	461 b.p.	60°C
S1P5	5' ^{1732}GGAGTAGTTCCCGAAGGACC^{1751} 3' ^{1967}TCTAGAATCCACGGGGTCTG^{1948}	236 b.p.	62°C
LPA1	5' ^{337}GCCACAGAATGGAACACAG^{355} 3' ^{819}GTAGAGGGGTGCCATGTTG^{801}	483 b.p.	62°C
LPA2	5' ^{342}GGCCTACCTCTTCCTCATGTT^{362} 3' ^{739}GCACATAGAAGAAAATTCGTG^{716}	398 b.p.	62°C
LPA3	5' ^{399}TTAGCTGCTGCCGATTTCTT^{418} 3' ^{647}ATGATGAGGAAGGCCATGAG^{628}	249 b.p.	62°C
GAPDH	5' ^{341}TTCTACAATGAGCTGCGTGTGGC^{363} 3' ^{757}CCTGCTTGCTGATCCACATCTGC^{735}	417 b.p.	62°C

RAECs expressed S1P1, 2, 3, 4 and LPA1, 3 (Figs. 1-3 and Table 6). The S1P4 and LPA1 receptors primers for rat were designed from known human and mouse sequences. Our results also indicated that mouse SV40 transformed endothelial pancreatic islet cells (MS1) expressed S1P1, 2, 3, 4 and LPA1, 2, 3 receptors. For comparative purposed, we also determined the LPL receptor expression profiles in mouse macrophage J774 cells. J774 cells expressed all eight LPL receptors (Figs. 1-3). In bovine cornea endothelial cells (BCECs) and bovine aortic endothelial cells (BAECs) express S1P1 and LPA1, 2, 3. However, bovine umbilical vein endothelial cells (BUVECs) expressed only LPA1, 2, 3 (Figs. 1-3 and Table 6). In conclusion, these data indicated that the different endothelial cells of different species expressed different LPLs receptors. Furthermore, endothelial cells isolated from the same sources in different species also have different LPLs receptors profiles (Table 7).

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Table 4. Rat LPLs receptors primer set.

Receptors	Primer sequence	Product size	Temp.
S1P1	5' ^{661}CTTCAGCCTCCTTGCTATCG^{680} 3' ^{1069}GCAGGCAATGAAGACACTCA^{1050}	429 b.p.	62°C
S1P2	5' ^{302}TTCTGGTGCTAATCGCAGTG^{321} 3' ^{996}GAGCAGAGAGTTGAGGGTGG^{977}	695 b.p.	60°C
S1P3	5' ^{125}GGGAGGGCAGTATGTTCGTA^{144} 3' ^{391}TGCTGATGAGAAAGGCAATG^{372}	267 b.p.	60°C
S1P4	5' CAAGACCAGCCGYGTSTAYG 3' CMGSWGGRCGTGGRGMCTTC	254 b.p.	62°C
S1P5	5' ^{469}TGTTCCTGCTCCTGGGTAGT^{488} 3' ^{1196}GTCTCGGTTGGTGAAGGTGT^{1177}	728 b.p.	60°C
LPA1	5' ^{202}ATTTCACAGCCCCAGTTCAC^{221} 3' ^{977}ACAATAAAGGCACCCAGCAC^{958}	776 b.p.	60°C
LPA2	5' ^{342}GGCCTACCTCTTCCTCATGTT^{362} 3' ^{739}GCACATAGAAGAAAATTCGTG^{716}	398 b.p.	62°C
LPA3	5' ^{1394}TGAGCCTCCATGTGTAGCTG^{1413} 3' ^{1787}AGCCACTAGCCTGGACTGAA^{1768}	394 b.p.	62°C
β-actin	5' ^{268}TTCTACAATGAGCTGCGTGTGGC^{290} 3' ^{1081}CCTGCTTGCTGATCCACATCTGC^{1059}	814 b.p.	62°C

Y = CT, S = GC, M = AC, W = AT, R = AG

Table 5. Bovine LPLs receptors primer set.

Receptor	Primer sequence	Product size	Temp.
S1P1	5' ^{1273}GACTCTGCTGGCAAATTCAAGCGAC^{1297} 3' ^{1624}ACCCTTCCCAGTGCATTGTTCACAG^{1600}	352 b.p.	62°C
S1P2	5' ^{551}CTCTCTACGCCAAGCATTATGTGCT^{575} 3' ^{1062}TCAGACCACCGTGTTGCCCTC^{1042}	512 b.p.	57.5°C
S1P3	5' ^{819}CAAAATGAGGCCTTACGACGCCA^{841} 3' ^{1519}TCCCATTCTGAAGTGCTGCGTTC^{1497}	701 b.p.	61.2°C
S1P4	5' ^{605}AGCCTTCTGCCCCTCTACTC^{624} 3' ^{943}GTAGATGATGGGGTTGACCG^{924}	394 b.p.	54.4°C
S1P5	5' ^{1732}GGAGTAGTTCCCGAAGGACC^{1751} 3' ^{1967}TCTAGAATCCACGGGGTCTG^{1948}	236 b.p.	59.7°C
LPA1	5' ^{733}CGGAGACTGACTGTCAGCAC^{752} 3' ^{1130}GGTCCAGAACTATGCCGAGA^{1111}	397 b.p.	62°C
LPA2	5' ^{342}GGCCTACCTCTTCCTCATGTT^{362} 3' ^{739}GCACATAGAAGAAAATTCGTG^{716}	398 b.p.	62°C
LPA3	5' ^{256}TTAGCTGCTGCCGATTTCTT^{275} 3' ^{647}ATGATGAGGAAGGCCATGAG^{628}	392 b.p.	62°C
GAPDH	5' ^{341}CCTTCATTGACCTTCACTACATGGTCTA^{363} 3' ^{757}GCTGTAGCCAAATTCATTGTCGTTACCA^{735}	417 b.p.	62°C

Table 6. The LPLs receptors expression profiles in different cells.

Species	Human			Bovine			Mouse		Rat
cells	Huvec	EAhy	HAE	BCEC	BAEC	Buvec	MS1	J774	RAEC
S1P1	∨	∨	∨	∨	∨		∨	∨	∨
S1P2							∨	∨	∨
S1P3	∨						∨	∨	∨
S1P4	∨			n.d.	n.d.	n.d.	∨	∨	∨
S1P5	∨	∨		n.d.	n.d.	n.d.		∨	
LPA1	∨	∨	∨	∨	∨	∨	∨	∨	∨
LPA2				∨	∨	∨	∨	∨	∨
LPA3	∨	∨	∨	∨	∨	∨	∨	∨	∨

n.d.= not determined

Table 7. The LPLs receptors expression profiles in different cells from same sources.

Sources	Umbilical vein EC			Aortic EC		
cells	Huvec	EAhy	Buvec	HAE	BAEC	RAEC
S1P1	∨	∨		∨	∨	∨
S1P2						∨
S1P3	∨					∨
S1P4	∨		n.d.		n.d.	∨
S1P5	∨	∨	n.d.		n.d.	
LPA1	∨	∨	∨	∨	∨	∨
LPA2			∨		∨	∨
LPA3	∨	∨	∨	∨	∨	∨

n.d.= not determined

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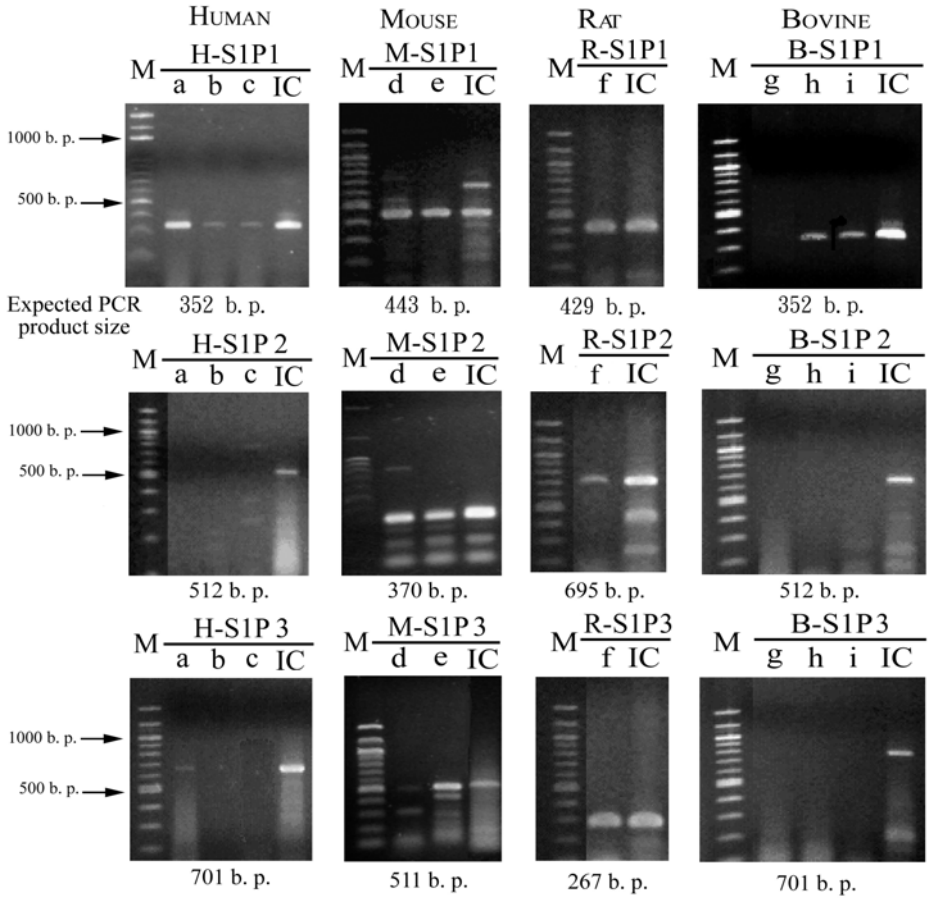


Fig. 1. S1P1 (upper panels), S1P2 (middle panels) and S1P3 (lower panels) expression profiles in endothelial cells isolated from human, mouse, rat and bovine origin. cDNAs were isolated from a: Human umbilical vein endothelial cells (HUVECs). b: HUVECs hybridoma cell line (EAhy926). c: Human aortic endothelial cells (HAECs). d: Mouse SV40 transformed endothelial pancreatic islet cells (MS1). e: Mouse macrophage (J774). f: Rat aortic endothelial cells (RAECs). g: Bovine umbilical vein endothelial cells (BUVECs). h: Bovine cornea endothelial cells (BCECs). i: Bovine aortic endothelial cells (BAECs) were subjected to PCR using specific primer sets. Predicted PCR product sizes were marked in the figure. M: 100 b.p. marker. IC: internal control for specific S1P receptors.

Partial sequences of Bovine and Rat LPLs receptors are highly conserved with other species

The PCR products of bovine LPLs receptors were gel eluted, sequenced and aligned by using ClustalW software. S1P1 PCR sequence from BAECs cDNA (Fig. 4), S1P4 PCR sequence from RAECs cDNA (Fig. 7), LPA2 PCR sequence from BUVECs cDNA (Fig. 8) and LPA3 PCR sequence from BCECs cDNA (Fig. 8) were conserved with known human, mouse and rat receptors sequences. To further confirm the negative expression of S1P2 and S1P3 receptors in bovine endothelial cells was not due to failure of PCR reactions, parallel PCR reactions using bovine genomic DNA as template were performed as control. S1P2 PCR product (Fig. 5) and S1P3 PCR product from bovine lung genomic DNA (Fig. 6) were sequenced and compared with sequences from other species. Both PCR products were highly conserved with known sequences.

DISCUSSION

S1P and LPA transfer their signals through the G protein-coupled receptors: S1P1-5 and LPA1-3. Several scientists have extensively investigated the trans-membrane signaling mechanism of the cloned LPLs receptors on both mammalian and non-mammalian cells (An et al., 1998a; An et al., 1998b; Lee et al., 1998; Goetzl et al., 1999; Lynch et al., 1999; Hla, 2001). The LPLs receptors share substantial homology in predicted amino acid sequences and therefore were considered to constitute a specific GPCR family. The similarity of amino acid sequence among S1P or LPA receptor isoforms is 46-54% or 32-36%, respectively (Chun et al., 2002).

Only a few reports discussed the LPLs receptors expressions on endothelial cells. We surmised that different endothelial cells might express different LPLs receptors and that would affect their functions. Therefore, to investigate the LPLs receptors

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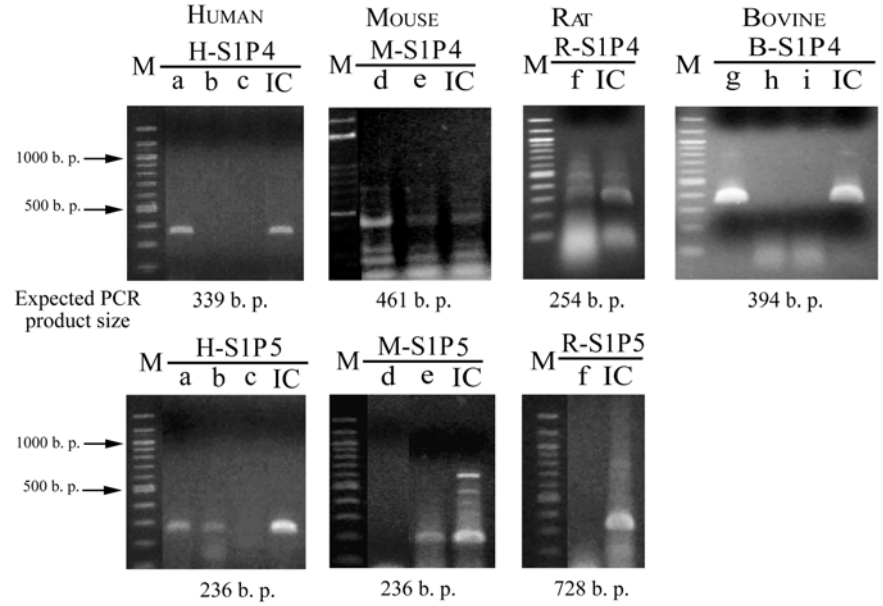


Fig. 2. S1P4 (upper panels) and S1P5 (lower panels) expression profiles in endothelial cells isolated from human, mouse, rat and bovine origin. cDNAs were isolated from a: Human umbilical vein endothelial cells (HUVECs). b: HUVECs hybridoma cell line (EAhy 926). c: Human aortic endothelial cells (HAECs). d: Mouse SV40 transformed endothelial pancreatic islet cells (MS1). e: Mouse macrophage (J774). f: Rat aortic endothelial cells (RAECs). g: Bovine umbilical vein endothelial cells (BUVECs). h: Bovine cornea endothelial cells (BCECs). i: Bovine aortic endothelial cells (BAECs) were subjected to PCR using specific primer sets. Predicted PCR product sizes were marked in the figure. M: 100 b.p. marker. IC: internal control for specific S1P receptors.

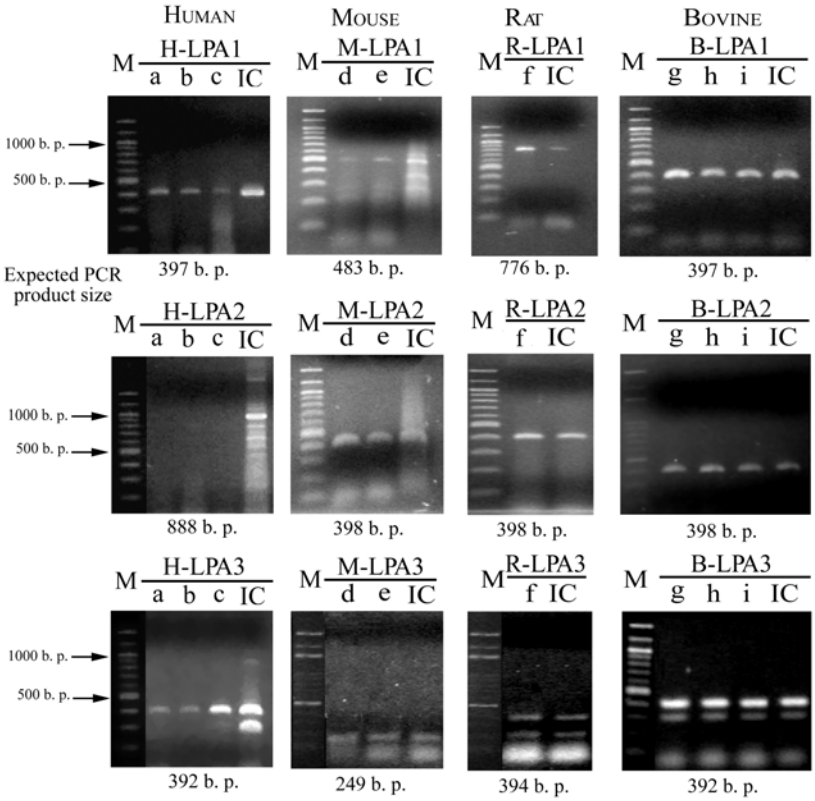


Fig. 3. LPA1 (upper panels), LPA2 (middle panels) and LPA3 (lower panels) expression profiles in endothelial cells isolated from human, mouse, rat and bovine origin. cDNAs were isolated from a: Human umbilical vein endothelial cells (HUVECs). b: HUVECs hybridoma cell line (EAhy926). c: Human aortic endothelial cells (HAECs). d: Mouse SV40 transformed endothelial pancreatic islet cells (MS1). e: Mouse macrophage (J774). f. Rat aortic endothelial cells (RAECs). g: Bovine umbilical vein endothelial cells (BUVECs). h: Bovine cornea endothelial cells (BCECs). i: Bovine aortic endothelial cells (BAECs) were subjected to PCR using specific primer sets. Predicted PCR product sizes were marked in the figure. M: 100 b.p. marker. IC: internal control for specific S1P receptors.

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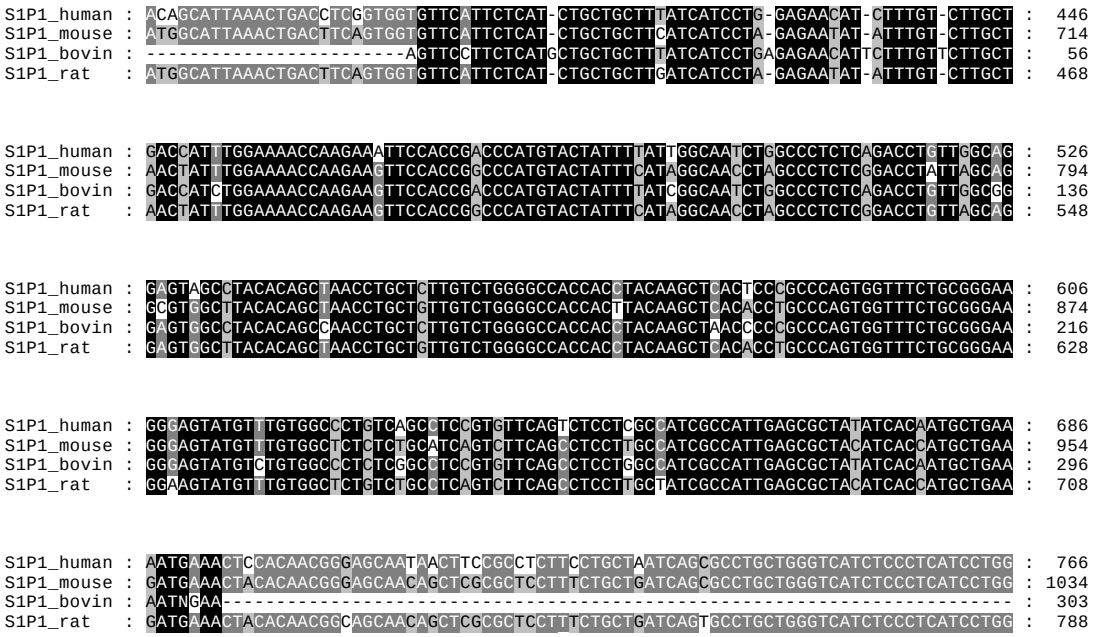


Fig. 4. The Bovine S1P1 receptor sequences were highly conserved with the mRNA sequences from other species. Human, mouse and rat known S1P1 sequences on NCBI were used to align with bovine S1P1 PCR product sequence using ClustalW software. The completely identical nucleic acids among four species were marked with black blocks. Light grey and dark grey blocks were used to mark the double and triple identity nucleic acids among four species.

expression on cells is relevant to understand the cellular physiological effects by S1P and LPA. RT-PCR might be a useful method to investigate their expression on cells while few LPLs receptors antibodies were available. To confirm our PCR results, all PCR experiment is run parallel with known internal control, such as genomic DNA or specific cDNA, using the same primer and temperature. Besides, we also used β-actin or GAPDH primers to confirm the cDNA quality.

Previous studies suggested that HUVECs and BAECs express S1P1 and 3 as well as LPA2, but not S1P2 and LPA 2 (Wang et al., 1999; Lee et al., 2000; Paik et al., 2001). Our results showed that HUVECs expressed S1P1, 3, 4, 5 and LPA1, 3 (Figs. 1-3) and BAECs expressed S1P1 and LPA1, 2, 3 but not S1P3 (Figs. 1 & 3). Based on our results, in the same species, different endothelial cells expressed different LPLs receptors patterns (Table 6). On the other hand, endothelial cell isolated form same origin form different species also have a distinct LPL receptor expression pattern (Table 7). Therefore, we concluded that endothelial cells isolated from different origins have distinct LPL receptor expression profiles.

In order to confirm the generated PCR products were LPLs receptors but not some non-specific contaminants, the PCR products generated by using specific designed bovine LPLs receptors primers were sequenced. These sequencing results suggested that bovine LPLs receptors mRNA sequences were highly conserved with other species, and the primers designed for bovine LPLs receptors were effective. These results support RT-PCR is a good method to detect LPLs receptors expressions in endothelial cells. These data also mean that without LPLs receptors antibodies, RT-PCR method for investigating LPLs receptors expressions was useful.

In our experiments, we could not detect specific PCR products for the bovine S1P5 receptor even using the bovine genomic DNA as a positive control (data not shown). We proposed that the primers designed for bovine S1P5 receptor using conserved regions of mRNA from known species were not effective. This result suggested that bovine S1P5 receptor might not be as conserved between species as other bovine LPL receptors do. PCR reactions using primer sets designed for bovine S1P4 did show an amplified product with predicted size. However, sequencing results suggested that the PCR product is not Edg receptors (D29972.1, AF367189). Therefore, we could not conclude if bovine endothelial cells express S1P4 or S1P5.

In this paper, by using conserved regions from known sequences, we generated partial sequences of rat S1P4 and LPA2 as well as bovine S1P1, S1P2, S1P3, S1P4, LPA2 and LPA3. Sequence alignment results showed high similarities between these sequences and published sequences from other

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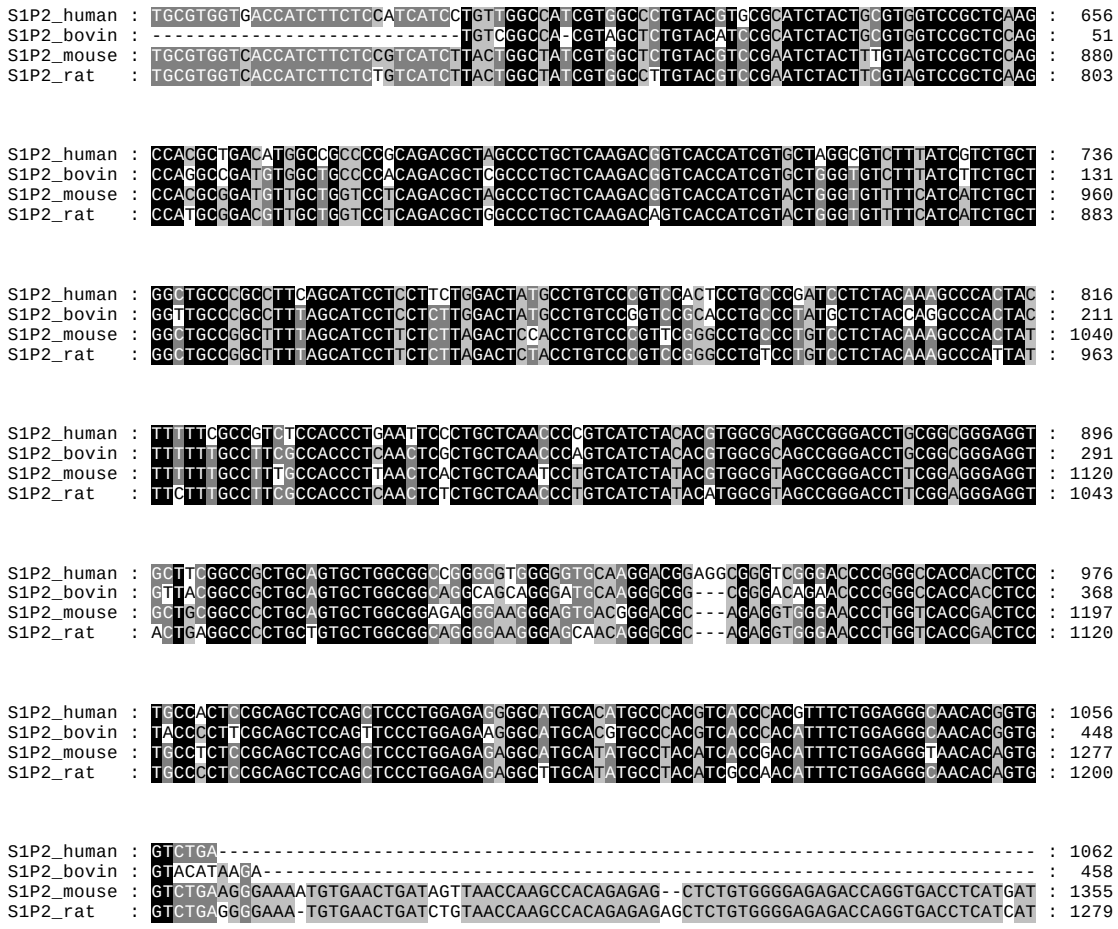


Fig. 5. The Bovine S1P2 receptor sequences were highly conserved with the mRNA sequences from other species. Human, mouse and rat known S1P2 sequences on NCBI were used to align with bovine S1P2 PCR product sequence using ClustalW software. The completely identical nucleic acids among four species were marked with black blocks. Light grey and dark grey blocks were used to mark the double and triple identity nucleic acids among four species.

species. Compare to human sequences, bovine and rat LPA2 are 85%, bovine LPA3 is 96%, bovine S1P1 is 93%, bovine S1P2 is 87%, bovine S1P3 is 90% and rat S1P4 is 84% homologous to human sequences. These results suggest that LPL receptors sequences are highly conserved among species.

In conclusion, different endothelial cells of different species expressed distinct LPLs receptors profiles. The expression profiles of different LPLs receptors among endothelial cells suggested that LPLs might have different physiological functions for these cells. Therefore, choosing an appropriate endothelial cell model to investigate physiological functions of S1P and LPA is crucial.

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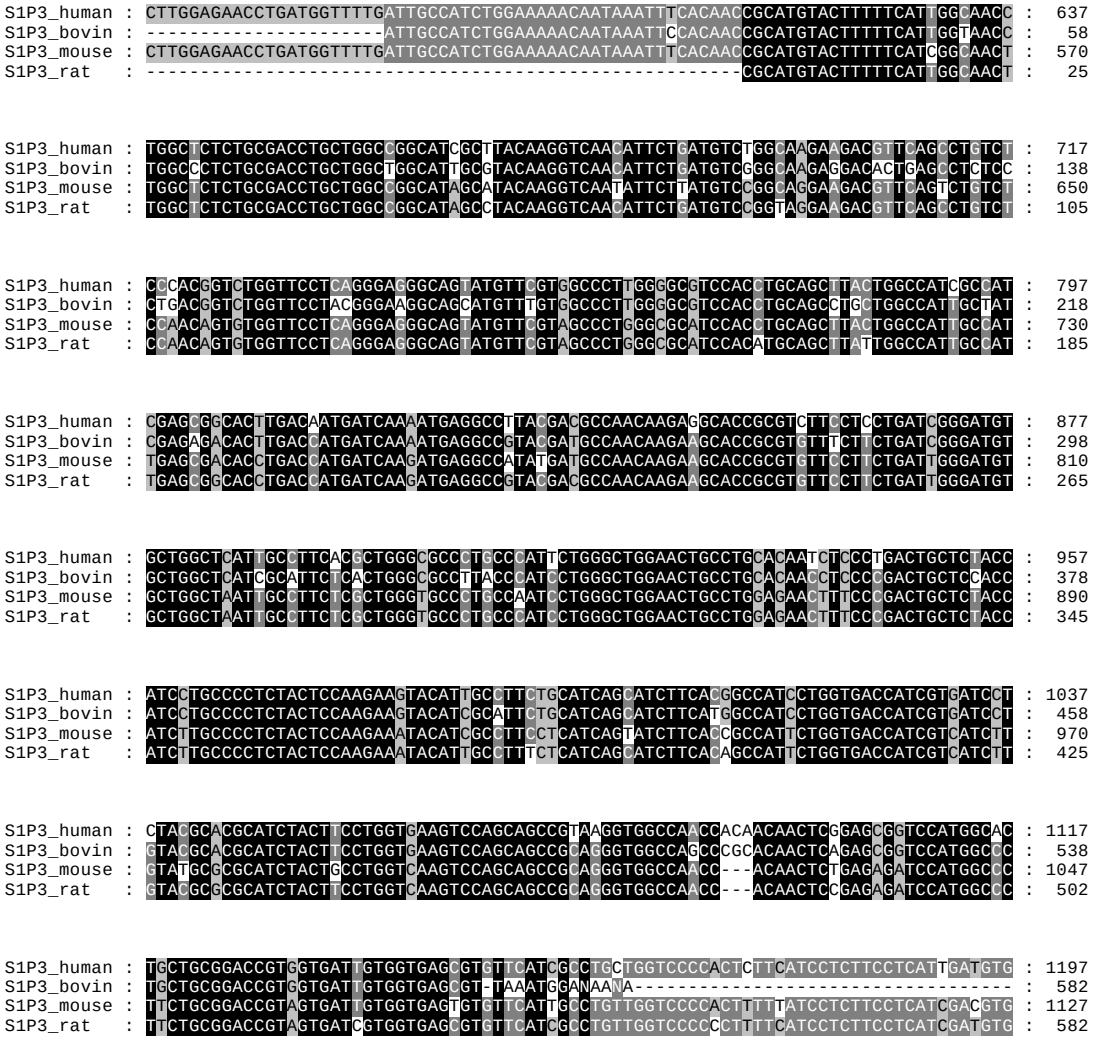


Fig. 6. The Bovine S1P3 receptor sequences were highly conserved with the mRNA sequences from other species. Human, mouse and rat known S1P3 sequences on NCBI were used to align with bovine S1P3 PCR product sequence using ClustalW software. The completely identical nucleic acids among four species were marked with black blocks. Light grey and dark grey blocks were used to mark the double and triple identity nucleic acids among four species.

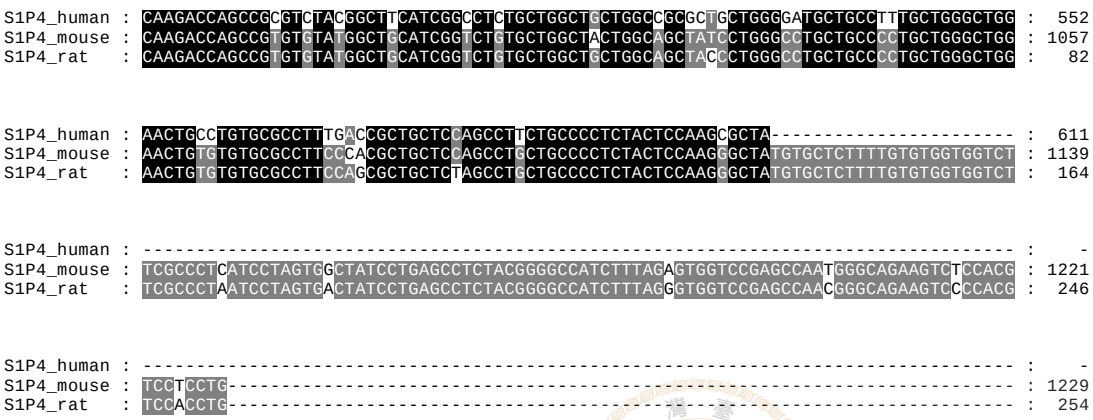


Fig. 7. The Bovine S1P4 receptor sequences were conserved with the mRNA sequences from other species. Human, mouse and rat known S1P4 sequences on NCBI were used to align with bovine S1P4 PCR product sequence using ClustalW software. The completely identical nucleic acids among four species were marked with black blocks. Light grey and dark grey blocks were used to mark the double and triple identity nucleic acids among four species.

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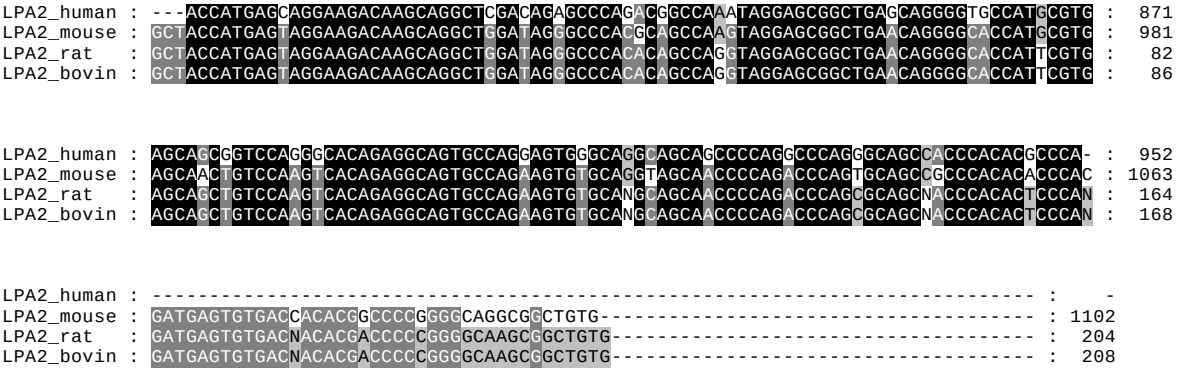


Fig. 8. The Rat and Bovine LPA2 receptor sequences were conserved with the mRNA sequences from other species. Human and mouse known LPA2 sequences on NCBI were used to align with rat and bovine LPA2 PCR product sequence using ClustalW software. The completely identical nucleic acids among four species were marked with black blocks. Light grey and dark grey blocks were used to mark the double and triple identity nucleic acids among four species.

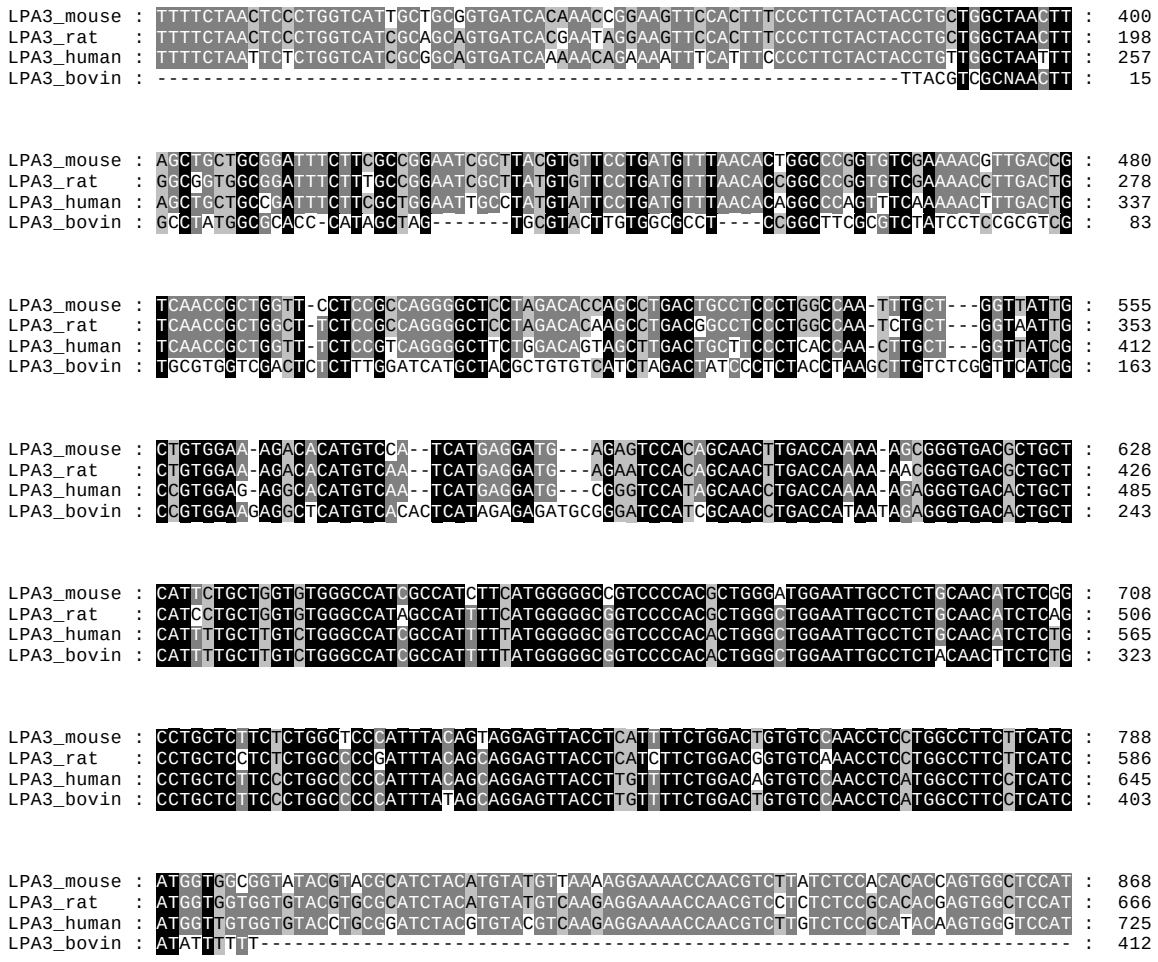


Fig. 9. The Bovine LPA3 receptor sequences were highly conserved with the mRNA sequences from other species. Human, mouse and rat known LPA3 sequences on NCBI were used to align with bovine LPA3 PCR product sequence using ClustalW software. The completely identical nucleic acids among four species were marked with black blocks. Light grey and dark grey blocks were used to mark the double and triple identity nucleic acids among four species.

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水解磷酸脂接受器於不同內皮細胞表達之特異性研究

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摘　　要

Sphingosine 1-phosphate（S1P）和 Lysophosphatidic acid（LPA）是兩種具有高度生物活性的水解磷酸脂，主要是經由被活化的血小板所釋放到血液之中。水解磷酸脂已知能夠透過與細胞膜上的一類 G 蛋白結合接受器結合，來引發細胞內一連串的訊息傳遞路徑，進而調控許多與內皮細胞相關的生理反應，例如：傷口癒合、血管新生、細胞附著等。此家族之 G 蛋白結合接受器，目前發現共有八種：其中 S1P1 到 S1P5 為 S1P 之接受器，而 LPA1 到 LPA3 則是 LPA 之接受器。水解磷酸脂接受器在細胞上的表現，已知會隨著不同細胞類型與細胞發育階段而不同。但對於不同來源之內皮細胞，其表面之水解磷酸脂接受器表現情形是否有所差異，則尚無相關之文獻探討。本研究針對由人類、小鼠、大鼠與牛四種物種之不同血管所分離出的血管內皮細胞，利用反轉錄－聚合酶鏈鎖反應（RT-PCR）來偵測不同水解磷酸脂接受器之表現情形。實驗結果顯示，不同物種來源與不同血管之血管內皮細胞上，所表現之水解磷酸脂接受器都有所不同，而此種差異可能直接影響到不同內皮細胞對水解磷酸脂之生理反應。

關鍵詞：LPA、S1P、血管內皮細胞、水解磷酸脂受器。

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