

Molecular cloning and endometrial expression of porcine high density lipoprotein receptor SR-BI during the estrous cycle and early pregnancy

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Abstract

During rapid development of the fetus, levels of high density lipoprotein (HDL) are elevated in pregnant women. The receptor for HDL, scavenger receptor class B type I (SR-BI), mediates selective cholesteryl ester uptake and is highly expressed in the human placenta. Because of the rapid growth of uterus during early pregnancy and differences in placentation between swine and humans, we hypothesized that SR-BI may be expressed in porcine endometrium to take up HDL cholesterol. The objectives of this study were to obtain the full coding region for porcine SR-BI, determine endometrial expression of SR-BI mRNA during the estrous cycle and early pregnancy, and map the gene. By iterative screening of a porcine expressed sequence tag library, we obtained the full coding region of SR-BI. Endometrial expression of SR-BI in White composite gilts ($n = 3-4$ each) was determined by Northern blotting on Days 10, 13, and 15 cyclic gilts and Days 10, 13, 15, 20, 30, and 40 pregnant gilts. In cyclic gilts, endometrial expression of SR-BI did not change between Days 10 and 13, but increased ($P < 0.01$) between Days 13 and 15. In pregnant gilts, endometrial expression of SR-BI increased ($P < 0.01$) between Days 10 and 13, remained elevated until Day 30, and decreased ($P = 0.015$) on Day 40. The SR-BI gene was mapped to 46.3 cM on chromosome 14. These results show that endometrial expression of SR-BI changes during the estrous cycle and early pregnancy, and suggest that SR-BI takes up HDL for endometrial development during early pregnancy.

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Keywords: cDNA; Endometrium; Gene expression; Mapping; mRNA

1. Introduction

The class B type I scavenger receptor (SR-BI), also known as CD36L1 and SCARB1, is a cell surface high density lipoprotein (HDL) receptor that mediates selective uptake of HDL cholesteryl esters (Steinberg, 1996; Rigotti et al., 1997; Azhar and Reaven, 2002). SR-BI HDL has been reported in the liver and steroidogenic tissues, including the adrenal gland and the ovary, of mice (Acton et al., 1996), rats (Landschulz et al., 1996; Johnson et al., 1998), and humans (Cao et al., 1997). SR-BI is highly expressed in the human placenta (Cao et al., 1997). The 85 kDa HDL-receptor SR-BI, which was originally designated as CD36 and LIMPII Analogous-1 (CLA-1) in humans (Calvo

and Vega, 1993), was detected on both brush border membranes and basal plasma membranes of human placenta (Lafond et al., 1999). However, SR-BI is expressed in low levels in both mouse (Acton et al., 1996) and hamster (Wyne and Woollett, 1998) placentas. SR-BI is expressed in high levels in the yolk sac of hamster (Wyne and Woollett, 1998). In mice, SR-BI was expressed in both the placenta and yolk sac beginning at Day E10 (Hatzopoulos et al., 1998). Thus, the presence of SR-BI in the placenta and its known function in transporting cholesteryl esters suggests the potential role in transferring HDL cholesterol from the maternal circulation to the placenta and fetus.

The uterus of pregnant pigs accommodates a large number of rapidly growing fetuses, therefore, the uterus has to grow rapidly during this period. Because SR-BI is suggested to deliver critical lipid nutrients to the ovary for membrane synthesis, steroid hormone production, or other processes (Miettinen et al., 2001), SR-BI may be expressed in the

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uterus to provide cholesterol for uterine membrane synthesis and maintenance. Uterine capacity is a critical component of litter size in pigs (Christenson et al., 1987). Genes that are expressed by the endometrium during early pregnancy may influence or be associated with differences in uterine capacity. SR-BI expression has not been studied in the pig.

The full coding regions for the SR-BI cDNA sequences of bovine (Rajapaksha et al., 1997), human (Calvo and Vega, 1993), mouse (Acton et al., 1996), and rat (GenBank accession no.: AB002151; Johnson et al., 1998) are known. However, the entire coding region of porcine SR-BI cDNA has not been reported. As a first step in determining whether the SR-BI gene may contribute to endometrial development, the objectives of this study were to clone the full coding region for SR-BI, examine SR-BI mRNA expression in porcine endometrium, and map the gene in the porcine genome.

2. Materials and methods

2.1. Isolation of porcine SR-BI cDNAs

Endometrium was collected on Day 30 from pregnant White composite gilts at slaughter and snap frozen in liquid nitrogen. Total RNA was isolated from the endometrium using the RNeasy kit (Qiagen, Santa Clarita, CA). To obtain a partial clone, reverse-transcription (RT) was performed using M-MLV reverse transcriptase (Promega, Madison, WI). Initially, 2 µg of total RNA and 2 µl of 10 µM reverse primer 4 (Table 1) was brought to 10 µl with water in a tube, then it was treated at 72 °C for 2 min and on ice for 2 min. After adding 4 µl of 5 × RT buffer, 2 µl of dNTPs, 2 µl of

reverse transcriptase, and 2 µl of water into the tube for the final 20 µl reaction volume, the tube was kept at 37 °C for 1 h. Then 1 µl of the resultant product was amplified with all possible pairs of forward (1 and 2) and reverse (1–4) primers (Table 1). After aligning the known bovine (Rajapaksha et al., 1997) and human (Calvo and Vega, 1993) SR-BI cDNAs, primers were designed based on the bovine SR-BI cDNA. Amplification with forward primer 1 and reverse primer 1 resulted in a 550 bp PCR product. This product was cloned into pCRII vector (Invitrogen, Carlsbad, CA) and sequenced in both directions. Primers based on the resulting sequence were then used to screen the “Meat Animal Research Center (MARC) 2 PIG” porcine expressed sequence tag (EST) library (Fahrenkrug et al., 2002) by PCR for the first round of screening. From the second round of screening, forward primer 1 and reverse primer 4 were used for PCR, to amplify a single 2129 bp product. Iterative screening of the “MARC 2 Pig” EST library revealed a cDNA clone (2670 bp) containing the full coding region of SR-BI (GenBank accession no.: AF467889). This clone was sequenced in both directions using vector (SP6 and T7) and the specific (Table 1) primers.

2.2. Northern blotting

Northern blot analysis was performed using 20 µg of total RNA from endometrium of White composite gilts on Days 10, 13, and 15 of the estrous cycle, and Days 10, 13, 15, 20, 30, and 40 of pregnancy ($n = 3-4$ each). Total RNA for Northern blotting was also isolated from the testis of mature White composite boar, and endometrium, ovary (corpus luteum, CL), adrenal gland, kidney, liver and spleen collected from a White composite gilt on Day 105 of pregnancy. All tissues were collected at slaughter. Animal procedures were reviewed and approved by the US Meat Animal Research Center Animal Care and Use Committee. Total RNA was electrophoresed in 1.5% agarose gels prepared in MOPS (3-[*N*-morpholino]propane-sulfonic acid)/formaldehyde buffer, and the gels were then blotted onto Hybond-N nylon membrane (Amersham Life Science, Buckinghamshire, England). Probe for SR-BI was generated by PCR with forward primer 1 and reverse primer 4 to amplify a 2129 bp product (248–2376 bp in Fig. 1), which includes most of the coding region and part of 3' UTR, in the presence of [³²P]CTP and using the 2670 bp SR-BI cDNA obtained from the EST library as template. Membranes were prehybridized for 30 min in Rapidhyb (Ambion, Austin, TX). Then 1 × 10⁶ cpm/ml of radiolabeled probe was added and blots were hybridized at 68 °C overnight. The membranes were washed with 2 × SSC, 0.1% SDS at 68 °C, and then with 0.1 × SSC, 0.1% SDS at 68 °C, and subjected to autoradiography. Later, the same membranes were stripped and hybridized with 18S RNA probe. Probe (123 bp product) for 18S RNA was generated in the presence of [³²P]dCTP by PCR using a plasmid containing a partial 18S cDNA as template, and specific forward and reverse

Table 1
Primers used in the characterization of the porcine scavenger receptor-BI cDNAs

Stage	Primer	Sequence		
Initial	Forward	F1	CATCAAGCAGGTCCTCAA	
		F2	CCTGTCCCCTTCTACCTCTCTGTCTAC	
	Reverse	R1	GAGGCCAGAATCGGAGTTGTTG	
		R2	AAGTGAGGATGGGAGAGAAACAAGG	
		R3	CGGGGTGTAGGGGCTGG	
		R4	GGATGATGTCAGTTTAGGCTGGAG	
	Primer walk	Forward	F3	GAACTTCCGGGCAGATGTGG
			F4	GATGACACCAGCTCCCAAGG
F5			TCCCAGCTGCCTCCATC	
Reverse		F6	GGGAGACCCTTCAGACATTC	
		R5	CTGGGTTTCAGGACTATGGC	
		Intron 10	Forward	GACAAACCGGGAAGATTGAAC
Reverse	CGCAGCCCAGAGCAAGGAG			
SNP	Forward	ACAGCCTCCAAAAGTCAAGTTC		
	Reverse	AGCCTGCAGGTGTCTTTATTC		
	Probe	GAGGATCTCCAAGAGGGTATG		

and 13 combined versus Day 15; (3) Day 20 versus Day 30; (4) Days 10, 13, and 15 combined versus Day 40; (5) Days 20 and 30 combined versus Days 10, 13, 15 and 40 combined.

2.4. Mapping

Forward (bases 1345–1365 in Fig. 1) and reverse (bases 1503–1485 in Fig. 1) primers were designed to amplify a portion of the porcine SR-BI gene corresponding to intron 10 of the human gene (Table 1). These primers were used to amplify by PCR the genomic DNA of eight parents from the MARC Swine Reference Population (Rohrer et al., 1994). Based on the human genomic sequence, this intron was expected to be approximately 800 bp (Cao et al., 1997). Agarose gel electrophoresis of the resulting porcine genomic products indicated that the corresponding region of the pig gene was approximately 900 bp. The amplified products from two pigs were subcloned into pCRII vector and sequenced using M13 vector primers (GenBank accession no.: AY323820). Sequences were assembled into contigs as described previously (Kim et al., 2003) and polymorphisms were identified. One of these polymorphisms, a C/T polymorphism, was informative in the litters from two F1 sows. An assay was designed to genotype this polymorphism using a MALDI-TOF mass spectrometer and Sequenom (San Diego, CA) genotyping technology. Forward, reverse, and probe primers corresponding to bases 476–497, 810–789,

and 618–599 (GenBank accession no.: AY323820), respectively, were used for the assay (Table 1).

3. Results

3.1. Isolation and characterization of porcine SR-BI cDNA

Nucleotide sequences and predicted amino acid sequences for putative porcine SR-BI cDNA (GenBank accession no.: AF467889) are shown in Fig. 1. The SR-BI cDNA contained 2670 bp, including a coding sequence of 1527 bp that encodes 509 amino acids. The 5' and 3' untranslated regions (UTR) of the porcine SR-BI cDNA were 143 and 998 bp, respectively. A portion of the 5' UTR of the porcine SR-BI cDNA sequence (70–143 bp) was 85% identical with a portion of the 5' UTR of the bovine SR-BI cDNA sequence (Rajapaksha et al., 1997) (35–106 bp; Fig. 2) and a smaller portion of the 5' UTR of the porcine SR-BI cDNA sequence (96–143 bp) was 83% identical with a portion of the 5' UTR of the human SR-BI cDNA sequence (CLA1; Calvo and Vega, 1993) (23–69 bp; Fig. 2). The sequence of the 3' UTR of the porcine SR-BI cDNA was 71% identical to that of bovine SR-BI. There were three conserved regions (more than 80% identical) in the 3' UTR between porcine and bovine sequences (Fig. 2).

5' UTR

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Human
(83%)                23  caggcgcggagccctgagcgcggcggtgggcccga-ggcgcgcagac 69
Porcine: 70  cctccgcccccatctcgttccctccacagggcggagcccagcgacctccgcgccccgcccagggcgcgagac 143
(85%)
Bovine: 35  cctccgtccccgtctcgggtccctccacagggcgggaaccccgcgagcaccgcg-gcgcgcg-gggcgcgagac 106
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3' UTR

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Porcine: 1674  ggtcccaaggatgccgagagccagcccagcctggcccctcagtcagaccagcttcccagcccctacaccccactt 1748
(87%)
Bovine: 1637  ggttccaaagatgccatcagccagccctgtctggccactcagccagaccaacttcccagcccctacaccccgtt 1711

1749  cttcaggactctctcagcggacagcccaccagtcccccaaatctga 1793
1712  cttcaggactcgtccgtggacagcccaccagtcaccaagtctga 1756

Porcine: 2327  tgggtttgggtgtccggggtcctccctccagcctaaactgacagcatcctgtgttctgagccggccactccctg 2401
(83%)
Bovine: 2261  tgggtttgggtgtcggggtccctccctccagcctaaactgacatcatcctgtatactgagctggctcacttccctg 2335

2402  gtgggggtgggtgggaggctgt 2422
2336  actggggtggcaggaagctgt 2356

Porcine: 2557  tggggcaagcctctgtctcttttctactggaagagaaa-tgaatttatcatctttaaataataataacaattga 2630
(91%)
Bovine: 2482  tgggacaagcctctgtctcttttctactggaagaaaactgaat--atcatctttaaataataattcacaattga 2554

2631  agtaataaaccttt 2644
2555  agtaataaaccttt 2568
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Fig. 2. Conserved 5' and 3' untranslated regions (UTR) of SR-BI sequences are listed. 5' UTR (96–143 and 70–143 bp) of the porcine SR-BI cDNA sequence is highly conserved (83 and 85%) compared to a part of 5' UTR (23–69 and 35–106 bp) of the human and bovine SR-BI cDNA, respectively. There are three conserved (more than 80% identical) regions between porcine and bovine sequences in the 3' UTR.

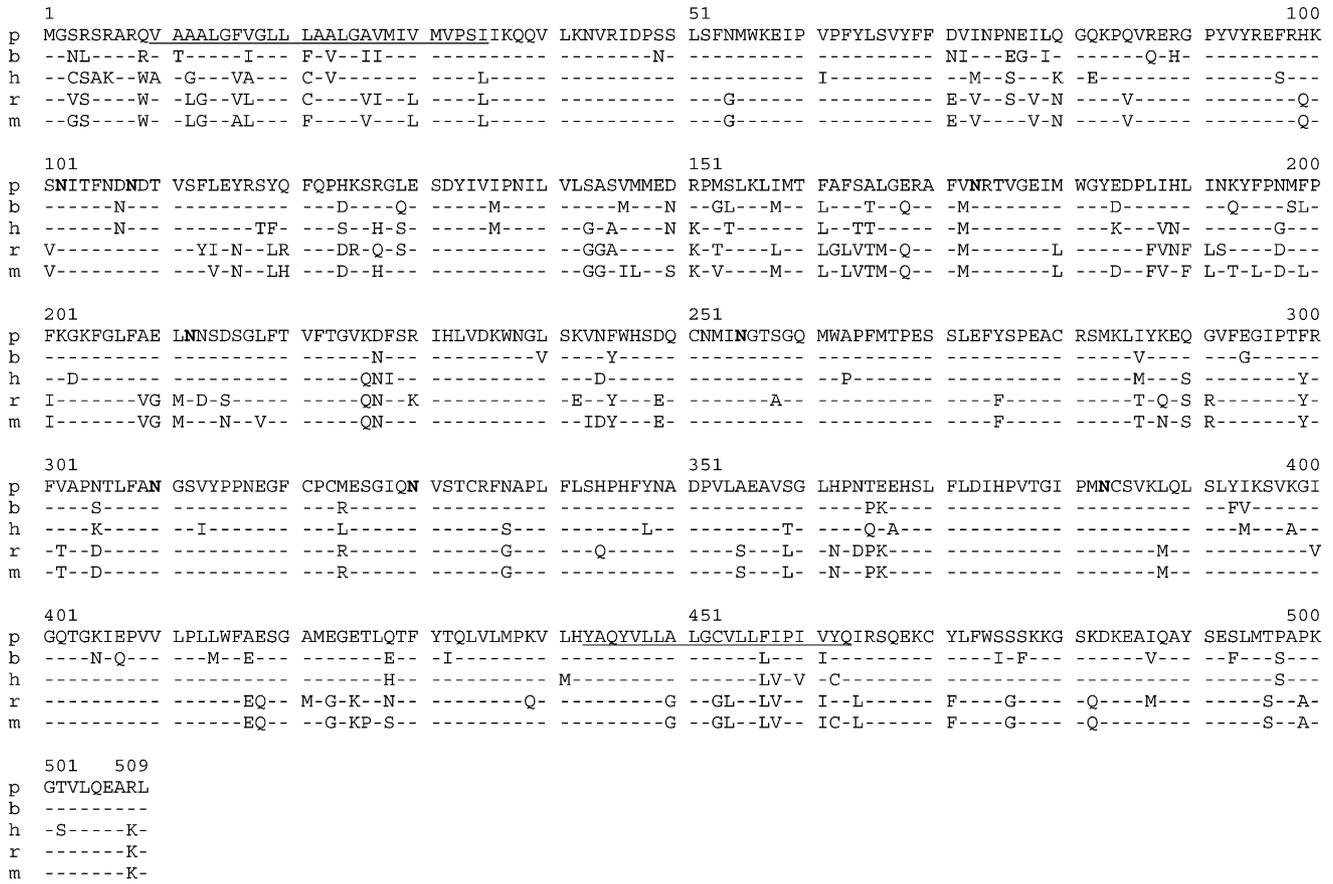


Fig. 3. A comparison of the predicted amino acid sequence for putative porcine SR-BI (p) with those of bovine (b), human (h), mouse (m), and rat (r) SR-BI sequences is shown. The two hydrophobic regions indicating transmembrane domains are underlined. Eight N-glycosylation sites in the extracellular domain are indicated by bold letters.

Percent identities between the amino acid sequences of porcine SR-BI with bovine (Rajapaksha et al., 1997), human (CLA1; Calvo and Vega, 1993), mouse (Acton et al., 1996), and rat (GenBank accession no.: AB002151) SR-BI were 88, 87, 80, and 79%, respectively. Alignment of the encoded amino acid sequences of porcine, bovine, human, and rat SR-BI indicates two hydrophobic regions, corresponding to transmembrane domains (Fig. 3). There are 5 highly conserved regions (aa 36–70, 90–112, 169–183, 200–285, 300–442), however, the importance of these regions has not been determined. Analysis of the sequence using Protein BLAST (NCBI) revealed a CD36 domain (aa 28–445) for scavenger receptors. Potential N-glycosylation sites are present at amino acid residues 102, 108, 173, 212, 255, 310, 330, and 383 (Figs. 1 and 3). All of the potential N-glycosylation sites are located in the extracellular domain and are conserved among the species compared (Fig. 3).

3.2. Expression of the SR-BI mRNA in endometrium and other tissues

Least-square means of densitometry units (\pm standard error means) for SR-BI mRNA in endometrium during the estrous cycle and pregnancy are illustrated in Fig. 4. A

representative autoradiograph of a Northern blot using endometrial total cellular RNA indicates SR-BI mRNA and 18S RNA bands (Fig. 4, top panel). A single SR-BI mRNA band of approximately 2.9 kb was observed. There was a status-by-day interaction ($P < 0.01$) in SR-BI mRNA expression. In cyclic gilts, endometrial expression of SR-BI mRNA did not change between Days 10 and 13, but increased ($P < 0.01$) between Days 13 (84.4 ± 10.8 arbitrary units) and 15 (151.7 ± 9.3). In pregnant gilts, endometrial expression of SR-BI mRNA increased ($P < 0.01$) between Days 10 (100.0 ± 9.3) and 13 (140.5 ± 9.3), remained elevated until Day 30 (157.5 ± 10.9), and decreased ($P = 0.015$) on Day 40 (113.4 ± 10.8). Expression of SR-BI mRNA was high in the adrenal gland and ovary (CL), and appeared to be slightly lower in the testis (Fig. 4, bottom panel). Liver, spleen, and endometrium appeared to have similar levels of SR-BI mRNA (Fig. 4, bottom panel). The expression of SR-BI mRNA in the kidney was low.

3.3. Mapping of the SR-BI gene

A C/T polymorphism, detected in two F1 sows, was genotyped across seven litters in the MARC Swine Reference Population, resulting in 25 informative meioses. This marker

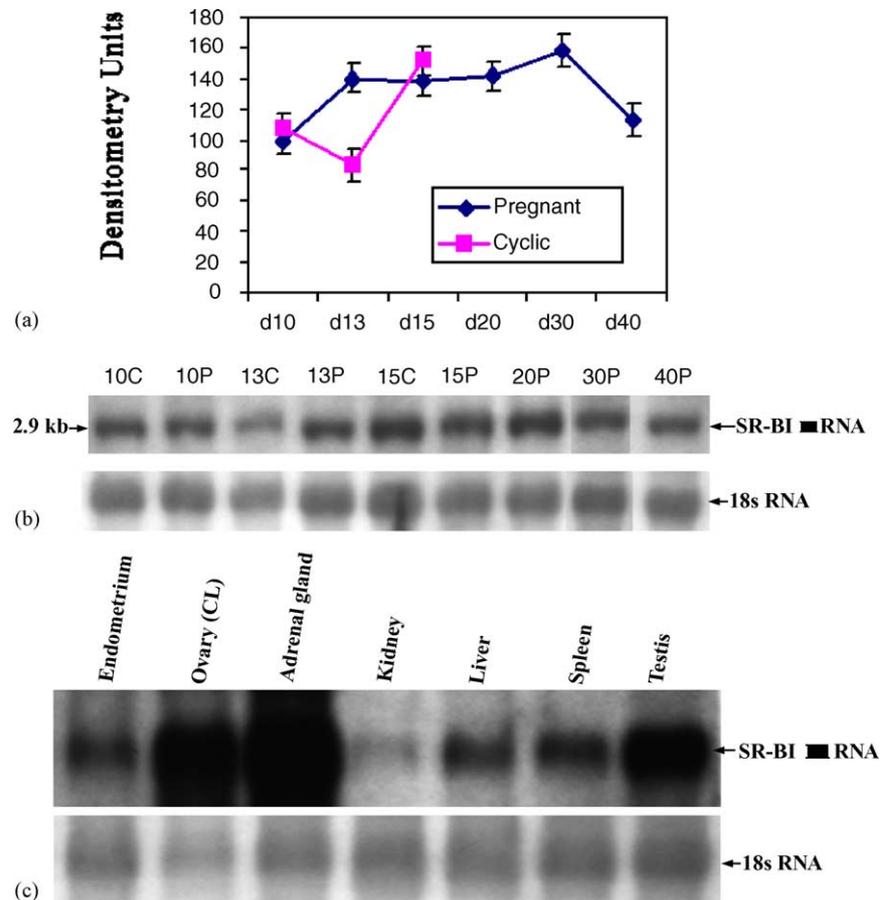


Fig. 4. Least-square means (\pm standard error means) of densitometry units for scavenger receptor class B type I (SR-BI) mRNA in endometrium during the estrous cycle and pregnancy are illustrated (a). In pregnant gilts, endometrial expression of SR-BI mRNA increased ($P < 0.01$) between Days 10 and 13, remained elevated until Day 30, and decreased ($P = 0.015$) on Day 40. These findings show that SR-BI mRNA expression is temporally regulated during early pregnancy. A representative autoradiograph of a Northern blot for SR-BI and matching 18S ribosomal RNA bands is also shown (b). Gels were loaded with total cellular RNA (20 μ g) from endometrium of cyclic (C) gilts on Days 10, 13, and 15, and pregnant (P) gilts on Days 10, 13, 15, 20, 30, and 40. Tissue distribution of SR-BI mRNA is shown (c). Gels were loaded with total cellular RNA (20 μ g) isolated from the testis of a mature White composite boar, and from endometrium, ovary (CL), adrenal gland, kidney, liver and spleen collected from a White composite gilt on Day 105 of pregnancy.

was mapped to 46.3 cM on chromosome 14, which is the same as marker SW210 on the current MARC swine chromosome 14 linkage map (<http://www.marc.usda.gov/>) using CRI-MAP. The most significant two-point linkage detected was with SW210 (LOD = 6.95).

4. Discussion

This is the first report of the full coding region of porcine SR-BI cDNA. The amino acid sequence of porcine SR-BI is more similar to bovine SR-BI than it is to human or rodent SR-BI. In pregnant gilts, endometrial expression of SR-BI mRNA was elevated on Day 13 and remained elevated until Day 30, while it was elevated on Day 15 in cyclic gilts. Finally, the SR-BI gene was mapped to 46.3 cM on chromosome 14 within a QTL associated with the last-lumbar back fat.

The sizes of the porcine 5' (143 bp) and 3' (998 bp) UTR obtained from this study were similar to those of the bovine

SR-BI cDNA (106 and 962 bp, respectively; Rajapaksha et al., 1997). In addition, sequence alignment showed high sequence identity between the porcine and the bovine SR-BI cDNAs in the 5' UTR. The 5' and 3' UTR sequences of the human (CLA-1; Calvo and Vega, 1993), mouse (GenBank accession no.: NM_016741), and rat SR-BI (GenBank accession no.: AB002151) cDNAs reportedly contain 69 and 967, 192 and 1125, and 202 and 1125 bp, respectively. The highly conserved regions in the 5' and 3' UTR between porcine and bovine SR-BI cDNAs suggest possible functional significance in those sequences.

The AATAAA sequence located 6 bases upstream from the polyA tail is likely to be the polyadenylation signal for porcine SR-BI cDNA. For bovine (Rajapaksha et al., 1997), mouse (GenBank accession no.: NM_016741), rat (GenBank accession no.: AB002151) and human (Calvo and Vega, 1993) SR-BI cDNAs, the locations of the polyadenylation signals of AATAAA were conserved and located in a similar position compared to that of porcine SR-BI.

Alignment of the encoded amino acid sequences of porcine, bovine, human, and rat SR-BI shows that the eight N-glycosylation sites in the extracellular domains are conserved (Fig. 3). At 227, the porcine sequence has Asp, while the rest of the species have Asn, giving an additional N-glycosylation site for other species. Rodents have an extra glycosylation site at 116. Potential intracellular phosphorylation sites for protein kinase C at 477 (Ser, except bovine) and at 481 (Ser) are conserved among the species compared, and they may be involved in the regulation of receptor activity (Johnson et al., 1998). Also conserved is a leucine zipper domain (aa. 427–455). A conservative change occurs at 508 (Arg) in porcine and bovine SR-BI, compared to Lys in other species for their putative peroxisomal targeting sequence (PTS1, aa 507–509; Johnson et al., 1998).

The size of SR-BI mRNA band in the pig is approximately 2.9 kb, which is larger than 2.4 kb (Landschulz et al., 1996) or 2.5 kb (Mizutani et al., 1997) in rats, and similar to 2.8 kb in humans (Cao et al., 1997). The porcine SR-BI cDNA sequence that we obtained (2760 bp) is close to the size of SR-BI mRNA band (approximately 2.9 kb). The single band that was obtained from different tissues in this study indicates that there is no differential splicing while differential splicing was reported in other species (Calvo and Vega, 1993; Webb et al., 1997).

The observed changes in SR-BI mRNA in cyclic and pregnant gilts show that the endometrial expression of SR-BI mRNA is differentially regulated in pregnant gilts compared to cyclic gilts. Elevated endometrial SR-BI of pregnant gilts may provide lipid nutrient to the developing endometrium. Endometrial expression of SR-BI mRNA may be affected by estrogen as previous reports in other systems indicate that estrogen may affect SR-BI gene expression. Estrogen treatment increased SR-BI in the adrenal gland and corpus luteum of the ovary, but reduced SR-BI in the liver of the rat (Landschulz et al., 1996). Estradiol increased SR-BI promoter activity in the human breast carcinoma MCF-7 cells and the rat luteal GG-CL cell line, which showed that estradiol can positively regulate SR-BI through estrogen receptors (Lopez et al., 2002).

Plasma and uterine luminal estrogens differ in cyclic and pregnant pigs (Geisert et al., 1990), and this may contribute to the differences in endometrial SR-BI mRNA expression. The elevation of SR-BI mRNA between Days 10 and 13 of pregnancy coincides with elevated uterine estrogen content on Day 11–12 of pregnancy from the conceptus (Geisert et al., 1990). However, it does not correlate with the declining uterine estrogen content on Day 14 (Geisert et al., 1990). Elevated SR-BI mRNA between Day 15 and 30 correlates with a second sustained increase of uterine estrogen after Day 14 (Geisert et al., 1990). Its decrease between Days 30 and 40 also correlates with estradiol concentrations in the uterine vein (Knight et al., 1977). The elevation of SR-BI mRNA between Days 13 and 15 of the estrous cycle correlates with the rise of plasma estradiol-17 β in cyclic gilts beginning from Day 15 (Geisert et al., 1990). Though es-

trogen receptor expression has been reported (Yang et al., 2003; Tarleton et al., 1998), whether SR-BI expression is regulated through estrogen receptor has not been studied in the porcine endometrium.

Expression of SR-BI mRNA or protein has been detected in the ovary or testis of various species (Acton et al., 1996; Cao et al., 1997; Johnson et al., 1998). In the ovary, the elevated level of progesterone in the plasma of the pig on Day 15 of pregnancy (Geisert et al., 1990) and SR-BI mRNA expression in the ovary (CL) described in this report suggest that SR-BI may be involved in steroidogenesis of the pig ovary. In the testis, rat Sertoli cells phagocytose apoptotic spermatogenic cells in primary cultures. SR-BI functions as a phosphatidyl serine receptor enabling them to recognize phosphatidyl serine exposed on the surface of degenerating spermatogenic cells and phagocytose apoptotic spermatogenic cells (Shiratsuchi et al., 1999). In addition, SR-BI mRNA is expressed in the developing mouse testes (Cao et al., 1999). These data indicate that SR-BI plays various roles in reproductive organs. SR-BI knockout mice have abnormal HDL, ovulate dysfunctional oocytes and are infertile (Miettinen et al., 2001), which further supports its role in reproduction. Furthermore, SR-BI is involved in the uptake of free cholesterol in the small intestine (Werder et al., 2001), showing SR-BI participates in the broad spectrum of physiological functions.

SR-BI is expressed in adipose tissue of the rat (Johnson et al., 1998). A QTL associated with the last-lumbar back fat was identified near 41 cM on swine chromosome 14 (95% confidence interval 24–63 cM, Rohrer and Keele, 1998). As SR-BI is involved in lipid transport and is mapped within the QTL, SR-BI may be associated with the last-lumbar back fat in pigs.

In conclusion, the full coding region of porcine SR-BI cDNA was obtained and sequenced. Temporal changes in SR-BI gene expression in the endometrium of pregnant gilts suggests that SR-BI may play a role in endometrial development during early pregnancy in swine. Understanding the mechanism of endometrial SR-BI expression may enable us to find ways to modulate endometrial development and increase uterine capacity in pigs.

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