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Review Article

Biological Functions of the Novel Collectins CL-L1, CL-K1, and CL-P1

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Collectins are characterized by a collagen-like sequence and a carbohydrate recognition domain and are members of the vertebrate C-type lectin superfamily. Recently, "novel collectins", different from "classical collectins" consisting of mannan-binding lectin (MBL) and surfactant proteins A and D (SP-A and SP-D), have been found by reverse genetics. These "novel collectins" consist of collectin liver 1 (CL-L1), collectin kidney 1 (CL-K1), and collectin placenta 1 (CL-P1) and are encoded by three separate genes. Experimental findings on human and animal collectins have shown that both novel collectins and classical collectins play an important role in innate immunity. Based on our recent results and those of others, in this paper, we summarize the new biological functions of these novel collectins in embryonic morphogenesis and development.

1. Introduction

Collectin is a C-type lectin conserved in animals from amphioxus to mammals. This protein possesses two characteristic structures, a collagen-like domain and a carbohydrate recognition domain (CRD). Recently, human collectins have been divided into two classes. The first is comprised of three "classical collectins", one harboring a mannan-binding lectin (MBL) [1], as well as two others, lung surfactant protein A (SP-A) [2] and lung surfactant protein D (SP-D) [3]. The second class is made up of three "novel collectins" consisting of collectin liver 1 (CL-L1) [4], collectin kidney 1 (CL-K1) [5], and collectin placenta 1 (CL-P1) [6]. MBL, an early discovered serum lectin, specifically recognizes carbohydrate moieties on microorganisms and is directly associated with innate immunity or acts through complement activation or opsonization via collectin receptors [7, 8]. Two surfactant proteins, SP-A and SP-D, also seem to play some role in host defence against various microbes [9, 10]. All three classical collectins are known to act in innate immunity as first-line host defence molecules.

Recently, cDNAs encoding the three new human collectins, CL-L1, CL-K1, and CL-P1, were cloned, and the properties of the products of these cDNAs were elucidated. These new collectins have been classified as "novel" and form three separate groups that differ from the earlier discovered classical ones. In this paper, the biological characteristics of these novel collectins will be presented.

2. Collectin Liver 1 and Kidney 1 (CL-L1, *COLEC10*, and CL-K1, *COLEC11*)

Our laboratory has cloned two cDNAs encoding these new proteins by screening human EST databases [4]. Cap site hunting was performed to determine the complete sequence, and it was designated collectin liver 1 (CL-L1) since mRNA of this collectin was first found in liver [4]. Secondly, the screening of the EST database using the amino acid sequence of the hCL-L1 CRD region discovered an EST clone, which appeared to encode the C-terminal region of a new human collectin. We isolated full-length cDNA by 5′-RACE and

cap site hunting using human kidney cDNA libraries [5]. This new human collectin from the kidney was termed collectin kidney 1 (CL-K1). CL-L1 cDNA consists of a nontranslated 75-nucleotide sequence at 5 prime followed by 831 nucleotides corresponding to the full-length protein and another nontranslated 759-nucleotide sequence with an"AATAA" incomplete polyadenylation signal at 3 prime [4]. The cDNA encoding CL-K1 had an open reading frame of 1383 bp, consistent with a sequence of 271 amino acid residues [5]. The cDNA contains a 240-nucleotide 5′-nontranslated sequence, followed by 813 nucleotides corresponding to the full protein, and a 330-nucleotide 3′-nontranslated sequence with an "AATAA" incomplete polyadenylation signal. The amino acid sequences deduced from the CL-L1 and CL-K1 cDNAs reveal that they have a collectin structure consisting of an N-terminal region with cysteine residues, a collagen-like region, a neck region, and a CRD. However, no CL-L1 hydrophobic signal peptide sequence was evident at the N-terminal region due to PSOLT (prediction of protein sorting signals and localization sites in amino acid sequences), thus N-terminal amino acid of the matured protein was not identified. It indicated that CL-L1 was presumed to be an intracellular protein. On the other hand, a hydrophobicity plot analysis showed that the product from CL-K1 cDNA contained a signal peptide consisting of 25 amino acids at its N-terminal end and a predicted mature protein of 246 amino acids.

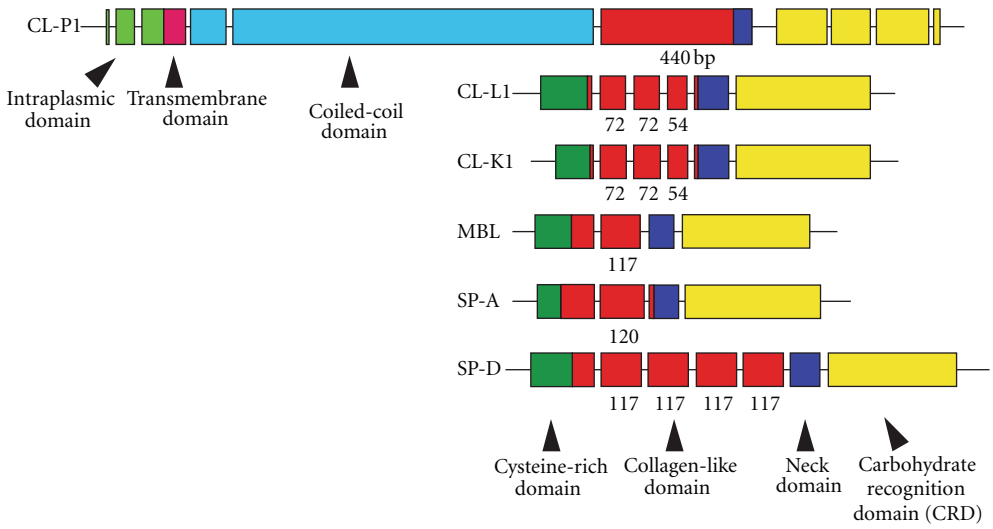
CL-L1 is a cytoplasmic-type collectin, whereas CL-K1 is a secreted-type collectin. Otherwise, their gene organizations are very similar (Figure 1). The sizes of the three exons encoding the collagen-like domain in CL-L1 and CL-K1 were 72 bp, 72 bp, and 54 bp, respectively. Their gene organization and the phylogenetic tree obtained using the amino acid sequence of the CRD indicated that these two genes evolved similarly from the same ancestor of the collectin gene [5]. Moreover, phylogenetic findings suggested that the two collectins should be considered to have emerged from a prototype collectin gene older than the classical type collectins MBL, SP-A, and SP-D.

Northern blot analysis of CL-L1 showed two bands with approximate lengths of 1.2 and 3.8 kb, which were mainly detected in human liver. The 1.2-kilobase mRNA was considerably more abundant than the 3.8-kilobase form. RT-PCR analyses also showed relatively high expression of hCL-L1 mRNAs in liver, placenta, adrenal gland, lung, small intestine, and prostate [4]. In the case of CL-K1, RT-PCR analyses showed high levels of mRNA in human and murine kidney, liver, fetal liver, small intestine, thymus, spinal cord, placenta, adrenal gland, and pancreas [5, 11]. The expression pattern of CL-K1 in murine tissues was confirmed by immunohistochemistry analysis [11].This ubiquitous expression is similar to that of CL-L1 but different from that of SP-A, SP-D, and MBL.

Immunoblot analyses using human liver demonstrated that CL-L1 was distributed to the cytoplasm, but not to the microsole or nucleus, and that the relative molecular size of the detected band corresponded to 40 kDa under reducing conditions. Immunofluorescence analyses of human primary hepatocyte cells also detected CL-L1 in the cytoplasm. In addition, in Chinese hamster ovary (CHO) cells transfected with pcDNA3.1/Myc-His A vector containing hCL-L1 cDNA, CL-L1 also localized in the cytoplasm. Human and mouse CL-L1 have a very unique C-terminal lysine cluster (Lys-Lys-Lys-Lys), whose four consecutive lysine residues are thought to act as a nuclear localization signal (NLS) in yeast aminoacyl tRNA synthetases [12]. This lysine cluster has also been observed in human cytoplasmic methionyl-tRNA synthetase (MRS) at the C-terminal end [13]. It is not known if the four lysine residues act as an NLS, translocating CL-L1 to the nucleus. Analysis of the cellular expression of CL-L1 protein is needed for characterizing its biological functions. Furthermore, a functional analysis should be performed to examine how the four lysine cluster might act as an NLS. Kawai and our group demonstrated the presence of murine CL-L1 and its mRNA in murine embryos and amnion, visceral yolk sac, and liver [14]. We observed that its mRNA expression increased with embryonic age and peaked dramatically on day 12 [14]. CL-L1 may be involved in embryonic development, but further investigations are required to elucidate the biological function of human and murine CL-L1. On the other hand, cellular localization of CL-K1 was performed by coupling it to a myc-His tag in CHO cells to create a fusion construct [5]. Immunostaining analysis using antimyc antibody and BODIPY TR as a specific Golgi marker and immunoblotting using an anti-carboxyl terminal myc tag antibody and anti-CL-K1CRD antibody revealed that CL-K1 is a secreted-type protein since it was detected to a much greater extent in culture medium than in cell lysates. The molecular size of CL-K1, which was purified as mannan-binding fraction from human serum and recombinant CL-K1 proteins without the myc-His tag, was determined to be approximately 34 kDa by western blot analysis [5]. We also determined partial amino acid sequences of native CL-K1 and recombinant CL-K1 and these sequences are Gln26-Pro-Ala-Gly-Asp-Asp-Ala32 and Lys119-Ala-Iso-Gly-Glu-Met-Asp-Asn-Gln-Val-Ser-Gln-Leu-The-Ser-Glu-Leu-Lys137, respectively. In addition, CL-K1 has other splicing variants with or without two collagen exons. Immunoblotting using recombinant CL-K1 proteins was performed and the complete size of CL-K1 with two collagen exons proved to be 34 kDa. The small CL-K1s were 30 and 28 kDa for one with exon and one without any exon, respectively.

The biosynthesis of a protein harboring a collagen triple helix is very unique, and it is essential that endoplasmic-reticulum-resistant chaperone heat-shock protein 47 (Hsp47) be used. Hsp47 binds to the collagen-related protein, which can correctly fold the triple-helical structure and give its stable form. Koide and our group reported that CL-L1 and CL-P1 are able to use this Hsp47 as a molecular chaperone [15]. In contrast, we found that MBL-2, MARCO, and adiponectin, which retain a collagen-related domain, do not seem to use this Hsp47. The usage of Hsp47 as a molecular chaperone is considered to be dependent on the presence of the Hsp47-binding motif in the protein, suggesting that CL-K1 has Hsp46-binding motif.

Sugar binding specificity was examined using recombinant hCL-L1 CRDs produced in *E. coli* [4]. Two recombinant



	MBL	SP-A	SP-D	CL-L1	CL-K1	CL-P1
N-terminal region	51%	37%	64%	72%	75%	95%
Collagen-like domain	75%	82%	79%	88%	94%	94%
Neck domain	18%	61%	57%	90%	93%	82%
CRD	60%	71%	76%	95%	97%	87%
Whole	60%	69%	75%	88%	92%	91%

Figure 1: The gene organization of human collectins and a comparison of the rates of the homology among the individual domains of human and murine collectins. In the gene organization schematic, the domain structure can be seen to consist of an intraplasmic domain, a transmembrane domain, a coiled-coil domain, a cysteine-rich domain, a collagenous domain, a neck domain, and a CRD. Collectin components are listed in the table as the N-terminal region, a collagen-like domain, a neck domain, a CRD, and the whole (domain).

hCL-L1 CRD fusion proteins, hCL-L1 CRD-histidine tag fusion protein (hCL-L1-CRDhis) and hCL-L1 CRD-maltose binding fusion protein (hCL-L1-CRDmal), were established. Sugar-blot analyses demonstrated that the two recombinant hCL-L1 CRDs possess Ca^{++}-dependent binding activity towards mannose. ELISA analyses using same sugar probes indicated that hCL-L1 has the ability to bind to D-mannose, L-fucose, and D-galactose > N-acetylglucosamine ≫ N-acetylgalactosamine. Kawai and our group reported that the recombinant mCL-L1 CRD exhibited properties similar to recombinant hCL-L1 [14]. Sugar-blot analyses demonstrated that CL-K1 has the ability to bind to L-fucose, weakly to D-mannose, but it could not bind to N-acetylgalactosamine, N-acetylglucosamine, or D-glucose [5]. We observed that CL-K1 Ca^{++} dependently bound to L-fucose-agarose, weakly to mannan-agarose and N-acetylglucosamine-agarose, but not at all to maltose-agarose or N-acetylgalactosamine-agarose. Unexpectedly, MBL could bind maltose-agarose and L-fucose-agarose, and bound even more strongly to mannan-agarose and N-acetylglucosamine-agarose [16]. Monosaccharide inhibition of CL-K1 binding to mannose-conjugated BSA was strongest with L-fucose and D-mannose followed by D-glucose and weakest by N-acetylglucosamine and D-galactose [17]. The five amino acid residues of the CRD, namely, the lectin frame, are responsible for its association with calcium ions in the binding of carbohydrates [18, 19]. The two amino acid residues in the CRD of CL-K1 are Glu232 and Asn234 (Glu-Pro-Asn) as in that of MBL. The lectin frame further supports its binding specificity for D-mannose, D-glucose, L-fucose and N-acetylglucosamine. However, CL-K1 binds strongly to L-fucose, but hardly to N-acetylglucosamine. We found that the four cysteines and other 14 amino acid residues, including Glu and Asn of CRD, were conserved among the three classical collectins [4]. The cysteines in the CRD are not for oligomerization, which is through those at the N-terminal of the collagen domain in MBL. However, the basic frame of MBL had two amino acid residues, Tyr231 and Asn233, which were substituted by Phe225 and Lys227 in the case of CL-K1. These substitutions might change the ability of the latter to bind to the *N*-acetylglucosamine [5].

Keshi and our group investigated the characteristics of recombinant CL-K1 binding to LPS and LTA from microbes using lectin blotting on ELISA plates [5]. We observed that CL-K1 and MBL could bind to LPS from *Escherichia coli* EH100 (Ra mutant) and *Escherichia coli* F583 (Rd mutant) and to mannan from *Saccharomyces cerevisiae.* CL-K1 also bound to LPS from *Klebsiella pneumoniae,* similar to the

binding of MBL to LPS of *Salmonella minnesota* and to LTA of *Staphylococcus aureus* and *Streptococcus pyogenes/sanguis*. We demonstrated that the binding of CL-K1 to LPS or LTA was very weak compared with that of MBL seen on lectin blotting. It is known that MBL recognizes carbohydrates on microbes via the CRD and can bind to them and activate the complement pathway [20–23]. These results all suggest that CL-K1 and CL-L1 might play some role in innate immunity.

Crystallography analysis and a point mutation study on the binding of MBL and MASP showed that the MASP-binding site was located in the collagen-like domain in MBL [24, 25]. Wallis et al. demonstrated that incorporation of the MASP-binding site (Hyp-Gly-Lys-Xaa-Gly-Pro) into SP-A, which does not activate a complement pathway, can activate complement pathway. However, its activation was uncontrolled and observed even in the absence of a carbohydrate molecule. The introduction of the additional amino acid sequence into the mutated SP-A might be needed for the physiological capacity for complement activation and deactivation. These results also revealed that CL-K1 like MBL, ficolin, and C1q possesses an MASP-binding site containing a protease-binding motif. After this experiment, Holmskov et al. demonstrated that CL-K1 could activate the complement pathway using recombinant CL-K1, and they confirmed its antimicrobial activity and tissue localization [17]. Furthermore, their findings with a small number of people showed that the blood concentration of CL-K1 was about 2 microgram/mL. We supposed several possibilities in the differences of ethnicity, sample sizes, and methods why its concentration was lower than that of our ELISA.

More recently, a surprising report has been published showed that the deletion of CL-K1 due to a gene mutation causes physical anomalies in humans [26]. This disease is a rare autosomal recessive genetic disorder which is designated as 3MC syndrome (Mingarelli-Malpuech-Michels-Carnevale syndromes). It is characterized by high arched eyebrows, ptosis, cleft palate, cleft lip, down-turned mouth, cognitive impairment, growth deficiency, and an insufficiency of craniosynostosis. Another related gene is the MASP-3 gene, which has the same mutation and deletion as the CL-K1 gene [26, 27]. Both genes were associated with a complement activation pathway so that mutations in these two genes might cause a hereditary disease. Yoshizaki and our group established a system for detecting CL-K1 in blood using several types of antibodies [28]. ELISA analysis of over 200 people showed that human blood concentration of CL-K1 was about 0.34 ± 0.13 micrograms/mL, lower than that of MBL. These two collectins are produced in liver, but their levels of expression have no correlation with each other. The concentration of CL-K1 is maintained at a low level without fluctuation, and its concentration is not dependent on gender or age. The ELISA system will be useful for elucidating the physiological and pathophysiological role of CL-K1 as well as in detecting the 3MC syndrome.

3. Collectin Placenta 1 (CL-P1, *COLEC12*)

Screening of the EST database using tblastn of the NCBI revealed a human cDNA clone from a human placenta cDNA library that encoded a collectin-like protein. This protein was also named collectin placenta 1 (CL-P1) since mRNA of this protein was abundant in placenta [6]. Its cDNA sequence revealed that CL-P1 has an open reading frame of 2226 base pairs encoding a sequence of 742 amino acids. We found that the deduced amino acid sequence represented a collectin protein containing a collagenous region and a CRD. In addition, it harbored an intracytoplasmic domain, transmembrane domain, and coiled-coil domains from the N-terminal end of its open reading frame. CL-P1 cDNA transfection resulted in immunofluorescent staining of the membranes of the transfected CHO cells using anti-myc tag and anti-CRD antibodies [6]. Moreover, CL-P1 was also detected on the surface of human umbilical vein endothelial cells (HUVEC) using anti-CRD antibody. These results strongly suggest that CL-P1 is localized on the cell membrane as a type II transmembrane protein. Results of immunocytochemistry demonstrated the expression of the CL-P1 protein in vascular endothelium and artery in murine and human [17, 29]. Its cellular expression in HUVEC and human umbilical artery endothelial cells (HUAEC) was shown by flow cytometry. However, THP-1, U937, and HL-60 treated with LPS, as well as macrophages showed no expression of this protein. SDS-PAGE and immunoblotting under reducing conditions revealed that CL-P1 had an approximate molecular mass of 140 kDa in CL-P1-cDNA-transfected CHO cells (CHO-CL-P1), HUVEC, and placental membrane extracts and that it might possess several N-glycosylation sites in its coiled-coil region and CRD. This was confirmed by deglycosylation treatment and in vitro translation. The higher molecular weight of the CL-P1 band under nonreducing conditions indicated that it was an oligomeric structure due to its collagen-like and coiled-coil helical domains.

The domain structure of CL-P1 seemed to be very similar to that of the scavenger receptor AI (SR-AI) judging from its predicted structural characteristics. We noted that the SR-cysteine-rich domain in SR-A was replaced by the CRD of CL-P1. To elucidate the function of CL-P1, the binding activity in CHO ldlA7 cells expressing CL-P1 and SR-BI was investigated using modified LDL. Results showed that CHO-CL-P1 cells bound to OxLDL but not to Ac-LDL or LDL, although CHO-SR-BI cells could bind to all LDLs (OxLDL, AcLDL, and LDL), while the control CHO ldlA7 cells did not bind to any of them [6]. The binding of OxLDL to CHO-CL-P1 cells was inhibited by the negative polycharged substances poly-I/G and dextran sulfate but not by LDL or AcLDL. Taken together, these results indicated that CL-P1 plays a role as a scavenger receptor. A previous study revealed that *E. coli* and *S. aureus*, but not *Saccharomyces cerevisiae* (yeast), could bind to SR-A and MARCO [30]. However, CHO cells expressing CL-P1 bound and phagocytosed yeast as well as *E. coli* and *S. aureus*. Recently, SR-AI knockout mice were shown to have an increased mortality from HSV and *Listeria* infections [31, 32]. These results indicate that scavenger receptors including CL-P1 may play a role in innate immunity, although CL-P1 is not a soluble-type collectin. In vascular spaces, CL-P1 might not only control the expansion of bacteria and yeasts but also regulate the amount of modified LDLs. Collagen-like domains, which have the

highest homology between amino acid sequences of human and murine CL-P1 and harbor positively charged islands, may play an important role in its binding to the negatively charged substances. SR-A, another scavenger receptor with a collagen-like domain, also maintains a strong homology between these charged sites of humans and mice. Mutation analysis showed that the positively charged islands formed binding sites for many ligands [33]. Jang and our group demonstrated the expression of CL-P1 in human and murine vascular endothelium by immunohistochemistry and real-time PCR analysis [29]. We characterized CL-P1-dependent phagocytosis in CL-P1-transfected cells as well as in human vascular endothelial cells. The use of small interfering RNAs of CL-P1 or other scavenger receptors showed that CL-P1 predominantly mediated phagocytosis of yeast in human vascular endothelium. CL-P1-dependent phagocytosis of fungi was also observed under culture conditions using serum and the efficiency of its phagocytosis in the culture medium increased several-fold when the serum was added. These results indicate that this phenomenon might occur under physiological conditions.

Yoshida et al. showed that CL-P1 was expressed in nurse-like cells and that recombinant CL-P1 fusion protein could bind to N-acetylgalactosamine. They demonstrated that this interaction was inhibited by N-acetylgalactosamine, T antigen (galactose-β(1-3)(N-acetylgalactosamine-αSer/Thr), and Tn antigen (N-acetylgalactosamine-α)1-O)Ser/Thr) [34]. Elola et al. reported that CL-P1 selectively bound to the sugar moiety designated Lewis^x (Le^x) [35], which is also known as stage specific antigen-1 (SSEA-1) having the structure of galactose-β(1-4)-fucose-α(1-3)-N-acetylglucosamine. They suggested that it might be expressed on glycoconjugates in human polymorphonuclear granulocytes (PMN) as well as in various tumors such as breast carcinoma, colon carcinoma, and Hodgkin's lymphoma. The glycan binding of CL-P1 and its distribution on vascular endothelial cells suggested that it might play a role in the binding of other cell adhesion molecules to the selectin in mediating the leukocyte-endothelial cell interaction. In addition, the Le^x antigen is present on various tumors and could mediate the adhesion of cancer cells to vascular endothelium through CL-P1. This interaction might be used to prevent tumor metastasis. Very recently, results of affinity chromatography on immobilized CL-P1 and mass spectrometry-based proteomics demonstrated that CL-P1 in vascular endothelial cells can bind and endocytose neutrophil granule glycoproteins [36]. These glycoproteins possess a multiclustered terminal Le^x group on a heterogenous mixture of branched glycans containing partially poly-N-acetyllactosamine residues. These results suggest that in vascular spaces, CL-P1 can act as a scavenger receptor in the clearance of glycoproteins released from neutrophils at sites of inflammation both locally and systemically.

CL-P1 was found to have a role in the scavenging of amyloid in Alzheimer's disease (AD) by Nakamura et al. [37], and the same group demonstrated that the expression of CL-P1 on amyloid-positive astrocytes and vascular endothelial cells was increased in a transgenic mouse AD model. A similar CL-P1 expression was observed in AD patients. These results suggest that CL-P1 might be involved in the clearance of amyloid molecules. Another study carried out haplotype analysis of the human CL-P1 gene of ten Japanese volunteers [38]. Of the six SNPs found (one in the 5′-upstream region, two in intron 2, one in exon 5, and two in exon 6), the two in exon 6 caused amino acid substitutions. These haplotype findings may be useful in studying disease associations.

Koyama and our group recently demonstrated the induction of CL-P1 under in vivo and in vitro conditions [39]. Hypoxia/reoxygenation stimulation induced CL-P1 mRNA and protein expression in HUVEC. Furthermore, the exposure of rat carotid artery to ischemic/reperfusion caused the upregulation of CL-P1 mRNA and protein, which peaked at 72 hours and at 7 days after treatment, respectively. The inducible expression of CL-P1 in vitro and in vivo due to oxidative stress is implicated in the development of endothelial dysfunction and chronic activation leading to the pathogenesis of atherosclerosis. Kim and our group carried out a promoter analysis using the 5′ flanking region of the CL-P1 gene [40]. We found that this gene is mainly regulated by three Sp1 (specificity protein-1) in the human CL-P1 proximal promoter and is a basic transcriptional factor. The third Sp1-binding site is controlled through some other factor, but not by an Sp1 protein, as observed with hydrogen peroxide treatment.

Very recently, Fukuda and our group described surprising findings in zebrafish [41]. We cloned the cDNA of a zebrafish CL-P1 (zCL-P1) which has 51% identity to human CL-P1 in its amino acid sequence. The mRNA expression of zCL-P1 was first observed 6 hours postfertilization (hpf), peaked at 24 hpf, and then decreased. The knockdown analysis of zCL-P1 demonstrated that the zebrafish embryos had severe morphological abnormalities such as short body length and defects in the dorsal aorta. The abnormalities were rescued by the injection of zCL-P1 or zVEGF mRNA. These results suggest a role for zCL-P1 in vasculogenesis and development during the early embryogenic stage of bony fish. Furthermore, in an in vitro study, it was revealed that cells transfected with the zCL-P1 gene could bind and endocytose modified LDL as well as microbes indicating that zCL-P1 plays a role in innate immunity as does human CL-P1. In addition, CL-P1 gene has high homology among humans, mice, and bony fish, especially in the intraplasmic region and collagen-like domain. The intraplasmic region containing the endocytosis motif, and collagen-like region has positively charged sites, suggesting that both regions might be important in signal transduction for development and for the scavenging functions of innate immunity.

4. The Biological Significance of Collectin Gene Evolution

Phylogenetic relationships among amino acid sequences of CRDs of various animal collectins have been demonstrated [5] in a presentation of the six classes of the collectin family: (1) the MBL group consisting of MBL, MBL-A, and MBL-C; (2) the SP-A group; (3) the SP-D group made up of SP-D, bovine CL-43, CL-46, and conglutinin; (4) the CL-P1

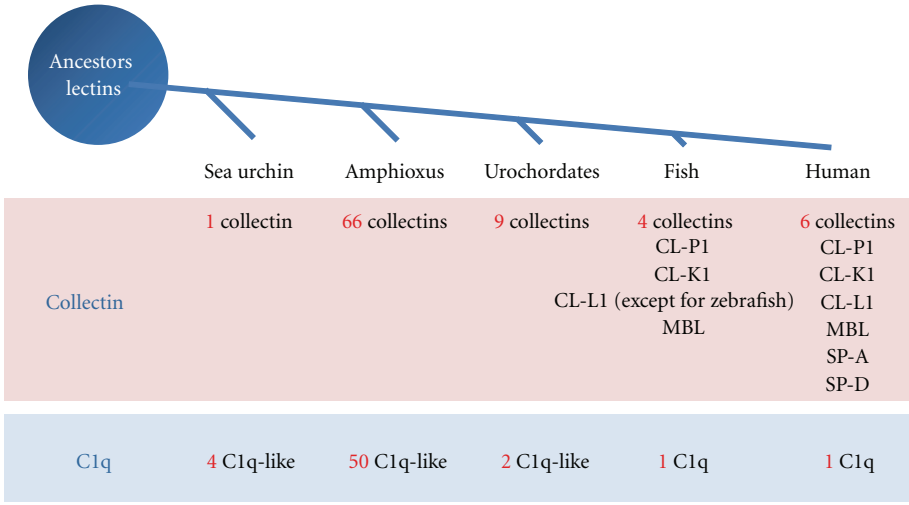


Figure 2: A schematic of the gene evolution of collectin and complement 1q (C1q). The extraordinary numbers of the two genes in amphioxus show that this life form has undergone enormous evolutionary changes since its emergence.

Table 1: Characteristics of classical collectin and novel collectin.

	Classical collectin			Novel collectin		
	MBL	SP-A	SP-D	CL-L1	CL-K1	CL-P1
Chromosomal location human	10q11.2	10q22.3	10q22.2-q23.1	8q23-q24.1	2p25.3	18pter-p11.3
mouse	MBL-A:14 MBL-C:19	14	14	15	12	18
HUGO *COLEC* no.	1	4	7	10	11	12
Localization	Secreted	Secreted	Secreted	Cytoplasmic	Secreted	Membrane
Tissue expression	Liver	Lung	Lung	Ubiquitous	Ubiquitous	Vascular endothelium
Sugar-binding specificities	GlcNAc > Fuc, Man	ManNAc > Fuc, Mal	Mal > Man, Glc	Man, Fuc, Gal	Fuc > Man	Gal, GalNAc, Le^x
Lectin frame	Y-N-EPN-E-	Y-N-EPA-E	Y-N-EPN-E	Y-N-EPS-E-	F-K-EPN-E	Y-N-QPD-E
Binding activity	Strong	Strong	Strong	Weak	Weak	Weak
Biological activities	Innate immunity	Innate immunity	Innate immunity homeostasis of surfactant	Unknown	Innate immunity? embryogenesis? structuraldevelopment?	Innate immunity scavenger receptor

group; (5) the CL-L1 group; (6) the CL-K1 group. All human gene names have been fixed by HUGO (Human Genome Organization). We have designated the novel collectin genes as *COLEC10* of the CL-L1 group, *COLEC11* of the CL-K1 group, and *COLEC12* of the CL-P1 group according to the order of their discovery.

Very recently, the entire genomes of amphioxus and urochordates have revealed that amphioxus have 66 collectin model genes and urochordates have 9 genes, whereas humans retain only 6 [42, 43]. Most primitive-type collectins can be observed in sea urchin. Screening of the entire genome was carried out using several fish. Fugu and medaka fish were found to have 4 collectin genes, while zebrafish has no CL-L1 gene. CL-L1, SP-A, and SP-D genes emerged during or after the evolution in bony fish. An analysis of chicken collectin genes showed that the three novel collectin genes CL-L1, CL-K1, and CL-P1 were conserved [44]. These results indicate that the evolution of collectin genes has occurred several times. The first occurrence was when amphioxus evolved from sea urchin, the second was when fish evolved from urochordates, and the third was when mammals evolved from fish (Figure 2). The figure demonstrates the numbers of collectin and complement 1q (C1q) at several stages of animal evolution. Ancestors such as the sea urchin might first use lectin for complement activation, whereas more advanced animals are able to use MBL, ficolin, and CL-K1 for the same activation. Finally, vertebrates can use not only the lectin pathway and these collectins but also C1q

and immunoglobulin in the classical pathway. The MASP gene is associated with the collectin gene and both evolved similarly, which may account for their similar involvement in the complement activation pathway. Since both genes have acquired an important role in vital homeostasis and embryonic development during gene evolution, they need to be conserved very strictly, and gene diversifications caused by a mutation, deletion, or insertion must be rejected. The classical collectin genes MBL, SP-A, and SP-D, which play a role in innate immunity, have all acquired genetic changes that fit their circumstances. On the other hand, CL-P1 is a membrane-type collectin as well as scavenger receptor, and its orthologue genes are highly conserved among various animals. These findings indicate that the genes essential for homeostasis must be conserved tightly if they are to survive evolutionary pressure (Figure 1).

5. Conclusion

In this review, the possible biological functions and characteristics of the novel collectins, CL-L1, CL-K1, and CL-P1, were summarized (Table 1). These new collectins function as innate immune molecules similar to the classical collectins based on in vitro studies. In addition, as seen in vivo, they might mediate development and vascular formation in bony fish, mice, and humans. Recently, it has been shown that CL-K1 is essential for developmental process during human embryogenesis. CL-L1 expressed in cytoplasm is a unique collectin with interesting biological functions. CL-K1 is a secreted protein, although it is very similar to CL-L1 with respect to its amino acid sequence, genomic organization, and gene development. The third collectin, CL-P1, was found to behave like a scavenger receptor in a cell expression study. Preliminary results suggest that CL-P1 might have an important role in development as well as in vascular homeostasis. At present, we are just at the beginning of collectin research and many questions may be resolved in the near future.

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References

[1] N. Kawasaki, T. Kaawasaki, and I. Yamashina, "Isolation and characterization of a mannan-binding protein from human serum," *The Journal of Biochemistry*, vol. 94, no. 3, pp. 937–947, 1983.

[2] R. T. White, D. Damm, and J. Miller, "Isolation and characterization of the human pulmonary surfactant apoprotein gene," *Nature*, vol. 317, no. 6035, pp. 361–363, 1985.

[3] A. Persson, D. Chang, K. Rust, M. Moxley, W. Longmore, and E. Crouch, "Purification and biochemical characterization of CP4 (SP-D), a collagenous surfactant-associated protein," *Biochemistry*, vol. 28, no. 15, pp. 6361–6367, 1989.

[4] K. Ohtani, Y. Suzuki, S. Eda et al., "Molecular cloning of a novel human collectin from liver (CL-L1)," *The Journal of Biological Chemistry*, vol. 274, no. 19, pp. 13681–13689, 1999.

[5] H. Keshi, T. Sakamoto, T. Kawai et al., "Identification and characterization of a novel human collectin CL-K1," *Microbiology and Immunology*, vol. 50, no. 12, pp. 1001–1013, 2006.

[6] K. Ohtani, Y. Suzuki, S. Eda et al., "The membrane-type collectin CL-P1 is a scavenger receptor on vascular endothelial cells," *The Journal of Biological Chemistry*, vol. 276, no. 47, pp. 44222–44228, 2001.

[7] D. L. Jack, N. J. Klein, and M. W. Turner, "Mannose-binding lectin: targeting the microbial world for complement attack and opsonophagocytosis," *Immunological Reviews*, vol. 180, pp. 86–99, 2001.

[8] M. Sumiya, M. Super, P. Tabona et al., "Molecular basis of opsonic defect in immunodeficient children," *The Lancet*, vol. 337, no. 8757, pp. 1569–1570, 1991.

[9] P. S. Kingma and J. A. Whitsett, "In defense of the lung: surfactant protein A and surfactant protein D," *Current Opinion in Pharmacology*, vol. 6, no. 3, pp. 277–283, 2006.

[10] C. Botas, F. Poulain, J. Akiyama et al., "Altered surfactant homeostasis and alveolar type II cell morphology in mice lacking surfactant protein D," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 95, no. 20, pp. 11869–11874, 1998.

[11] W. Motomura, T. Yoshizaki, K. Ohtani et al., "Immunolocalization of a novel collectin CL-K1 in murine tissues," *The Journal of Histochemistry and Cytochemistry*, vol. 56, no. 3, pp. 243–252, 2008.

[12] P. Schimmel and C. C. Wang, "Getting tRNA synthetases into the nucleus," *Trends in Biochemical Sciences*, vol. 24, no. 4, pp. 127–128, 1999.

[13] Y. G. Ko, Y. S. Kang, E. K. Kim, S. G. Park, and S. Kim, "Nucleolar localization of human methionyl-tRNA synthetase and its role in ribosomal RNA synthesis," *The Journal of Cell Biology*, vol. 149, no. 3, pp. 567–574, 2000.

[14] T. Kawai, Y. Suzuki, S. Eda et al., "Molecular cloning of mouse collectin liver 1," *Bioscience, Biotechnology and Biochemistry*, vol. 66, no. 10, pp. 2134–2145, 2002.

[15] T. Koide, Y. Nishikawa, S. Asada et al., "Specific recognition of the collagen triple helix by chaperone HSP47: II. The HSP47-binding structural motif in collagens and related proteins," *The Journal of Biological Chemistry*, vol. 281, no. 16, pp. 11177–11185, 2006.

[16] K. Ohtani, Y. Suzuki, S. Eda et al., "High-level and effective production of human mannan-binding lectin (MBL)in chinese hamster ovary (CHO) cells," *Journal of Immunological Methods*, vol. 222, no. 1-2, pp. 135–144, 1999.

[17] S. Hansen, L. Selman, N. Palaniyar et al., "Collectin 11 (CL-11, CL-K1) is a MASP-1/3-associated plasma collectin with microbial-binding activity," *Journal of Immunology*, vol. 185, no. 10, pp. 6096–6104, 2010.

[18] W. I. Weis, R. Kahn, R. Fourme, K. Drickamer, and W. A. Hendrickson, "Structure of the calcium-dependent lectin domain from a rat mannose-binding protein determined by

MAD phasing," *Science*, vol. 254, no. 5038, pp. 1608–1615, 1991.

[19] W. I. Weis, K. Drickamer, and W. A. Hendrickson, "Structure of a C-type mannose-binding protein complexed with an oligosaccharide," *Nature*, vol. 360, no. 6400, pp. 127–134, 1992.

[20] M. Devyatyarova-Johnson, I. H. Rees, B. D. Robertson, M. W. Turner, N. J. Klein, and D. L. Jack, "The lipopolysaccharide structures of Salmonella enterica serovar typhimurium and Neisseria gonorrhoeae determine the attachment of human mannose-binding lectin to intact organisms," *Infection and Immunity*, vol. 68, no. 7, pp. 3894–3899, 2000.

[21] A. S. Swierzko, M. Cedzyński, T. Kirikae et al., "Role of the complement-lectin pathway in anaphylactoid reaction induced with lipopolysaccharide in mice," *European Journal of Immunology*, vol. 33, no. 10, pp. 2842–2852, 2003.

[22] M. Matsushita, Y. Endo, and T. Fujita, "MASP1 (MBL-associated serine protease 1)," *Immunobiology*, vol. 199, no. 2, pp. 340–347, 1998.

[23] S. Thiel, T. Vorup-Jensen, C. M. Stover et al., "A second serine protease associated with mannan-binding lectin that activates complement," *Nature*, vol. 386, no. 6624, pp. 506–510, 1997.

[24] U. V. Girija, C. Furze, J. Toth et al., "Engineering novel complement activity into a pulmonary surfactant protein," *The Journal of Biological Chemistry*, vol. 285, no. 14, pp. 10546–10552, 2010.

[25] R. Wallis, D. A. Mitchell, R. Schmid, W. J. Schwaeble, and A. H. Keeble, "Paths reunited: initiation of the classical and lectin pathways of complement activation," *Immunobiology*, vol. 215, no. 1, pp. 1–11, 2010.

[26] C. Rooryck, A. Diaz-Font, D. P. S. Osborn et al., "Mutations in lectin complement pathway genes COLEC11 and MASP1 cause 3MC syndrome," *Nature Genetics*, vol. 43, no. 3, pp. 197–203, 2011.

[27] A. Sirmaci, T. Walsh, H. Akay et al., "MASP1 mutations in patients with facial, umbilical, coccygeal, and auditory findings of carnevale, malpuech, OSA, and michels syndromes," *American Journal of Human Genetics*, vol. 87, no. 5, pp. 679–686, 2010.

[28] T. Yoshizaki, K. Ohtani, W. Motomura et al., "Comparison of human blood concentrations of collectin kidney 1 and mannan-binding lectin," *The Journal of Biochemistry*, vol. 151, no. 1, pp. 57–64, 2012.

[29] S. J. Jang, K. Ohtani, A. Fukuoh et al., "Scavenger receptor collectin placenta 1 (CL-P1) predominantly mediates zymosan phagocytosis by human vascular endothelial cells," *The Journal of Biological Chemistry*, vol. 284, no. 6, pp. 3956–3965, 2009.

[30] A. Palecanda, J. Paulauskis, E. Al-Mutairi et al., "Role of the scavenger receptor MARCO in alveolar macrophage binding of unopsonized environmental particles," *Journal of Experimental Medicine*, vol. 189, no. 9, pp. 1497–1506, 1999.

[31] H. Suzuki, Y. Kurihara, M. Takeya et al., "A role for macrophage scavenger receptors in atherosclerosis and susceptibility to infection," *Nature*, vol. 386, no. 6622, pp. 292–296, 1997.

[32] H. Suzuki, Y. Kurihara, M. Takeya et al., "The multiple roles of macrophage scavenger receptors (MSR) in vivo: resistance to atherosclerosis and susceptibility to infection in MSR knockout mice," *Journal of Atherosclerosis and Thrombosis*, vol. 4, no. 1, pp. 1–11, 1997.

[33] T. Doi, M. Kurasawa, K. I. Higashino et al., "The histidine interruption of an α-helical coiled coil allosterically mediates a pH-dependent ligand dissociation from macrophage scavenger receptors," *The Journal of Biological Chemistry*, vol. 269, no. 41, pp. 25598–25604, 1994.

[34] T. Yoshida, Y. Tsuruta, M. Iwasaki, S. Yamane, T. Ochi, and R. Suzuki, "SRCL/CL-P1 recognizes GalNAc and a carcinoma-associated antigen, Tn antigen," *The Journal of Biochemistry*, vol. 133, no. 3, pp. 271–277, 2003.

[35] M. T. Elola, M. I. Capurro, M. M. Barrio et al., "Lewis x antigen mediates adhesion of human breast carcinoma cells to activated endothelium. Possible involvement of the endothelial scavenger receptor C-type lectin," *Breast Cancer Research and Treatment*, vol. 101, no. 2, pp. 161–174, 2007.

[36] S. A. Graham, A. Antonopoulos, P. G. Hitchen et al., "Identification of neutrophil granule glycoproteins as Lewis x-containing ligands cleared by the scavenger receptor C-type lectin," *The Journal of Biological Chemistry*, vol. 286, no. 27, pp. 24336–24349, 2011.

[37] K. Nakamura, W. Ohya, H. Funakoshi et al., "Possible role of scavenger receptor SRCL in the clearance of amyloid-β in Alzheimer's disease," *The Journal of Neuroscience Research*, vol. 84, no. 4, pp. 874–890, 2006.

[38] H. Ohmori, Y. Makita, M. Funamizu et al., "Haplotype analysis of the human collectin placenta 1 (hCL-P1) gene," *The Journal of Human Genetics*, vol. 48, no. 2, pp. 82–85, 2003.

[39] S. Koyama, K. Ohtani, J. Fukuzawa et al., "The induction of human CL-P1 expression in hypoxia/reoxygenation culture condition and rat CL-P1 after ischemic/reperfusion treatment," *Biochimica et Biophysica Acta*, vol. 1810, no. 9, pp. 836–842, 2011.

[40] Y. U. Kim, K. Ohtani, K. Mori, S. J. Jang, Y. Suzuki, and N. Wakamiya, "Gene regulation function of the three specificity protein-l (Sp1) within the human collectin placenta-1 proximal promoter," *Genes and Genomics*, vol. 33, no. 3, pp. 273–281, 2011.

[41] M. Fukuda, K. Ohtani, S.-J. Jang et al., "Molecular cloning and functional analysis of scavenger receptor zebrafish CL-P1," *Biochimica et Biophysica Acta*, vol. 1810, no. 12, pp. 1150–1159, 2011.

[42] N. H. Putnam, T. Butts, D. E. K. Ferrier et al., "The amphioxus genome and the evolution of the chordate karyotype," *Nature*, vol. 453, no. 7198, pp. 1064–1071, 2008.

[43] P. Dehal, Y. Satou, R. K. Campbell et al., "The draft genome of Ciona intestinalis: insights into chordate and vertebrate origins," *Science*, vol. 298, no. 5601, pp. 2157–2167, 2002.

[44] A. Hogenkamp, M. Van Eijk, A. Van Dijk, A. J. A. M. Van Asten, E. J. A. Veldhuizen, and H. P. Haagsman, "Characterization and expression sites of newly identified chicken collectins," *Molecular Immunology*, vol. 43, no. 10, pp. 1604–1616, 2006.