Ficolins

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Ficolins are soluble pattern-recognition molecules of the innate immunity. Ficolins consist of a collagen-like domain, a neck region and a fibrinogen-like domain that binds specific molecular structures of pathogens and the body's own damaged cells by preferably recognizing acetylated compounds. Humans have three homologous proteins, H-ficolin (or ficolin-3), L-ficolin (or ficolin-2) and M-ficolin (or ficolin-1). The first two ficolins are circulating in blood whereas ficolin-1 is locally secreted. Ficolins activates the lectin complement pathway, which results in neutralization of pathogens, thus ficolins play important roles in innate immunity, both to protect from infection as well as to maintain tissue health.

Advanced article

Article Contents

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Introduction

Ficolins are primordial proteins of the innate immune system that function in the maintenance of healthy tissue and as a first-line defense system against infection. The innate immune system is well orchestrated, with numerous other molecules and cellular components (Hoffmann *et al*., 1999). An essential advantage of innate immunity over adaptive immunity is that all the cells and molecules of the innate immune system exist and are maximally functional in the healthy state and do not require antigen-specific antibodies, which require up to a few weeks of lag time to reach their maximum levels. Pattern-recognition molecules of the innate immune system are capable of instant recognition of abnormal chemical epitopes, including invasive pathogens as well as altered self molecules, such as are exposed on dying cells, within a host that is otherwise healthy. In this process, ficolins may function by aggregating molecular targets, in addition to their ability to initiate another important innate immune mechanism, the lectin pathway complement (Fujita *et al*., 2004). **See also**: Collectins; Complement; Innate Immune Mechanisms: Nonself Recognition; Lectins; Pattern Recognition Receptor

Ficolins consist of a collagen-like domain at the *N*-terminus, linked by a neck region to a fibrinogen (FBG)-like domain at the *C*-terminus. In terms of their function and structure, ficolins are very similar to collectins, with the collectin carbohydrate-recognition domain (CRD) substituting for the ficolin FBG-like domain. The FBG-like domain selectively recognizes specific molecules, including certain acetylated compounds and carbohydrates that are exposed on the target surface. The human ficolin family has three members, H-ficolin, L-ficolin and M-ficolin, also known as ficolin-3, -2 and -1, respectively. The ficolins are themselves members of the larger family of C-type lectins, which requires calcium for ligand binding and includes collectins, mannose receptor, sialic acid-binding immunoglobulin-like lectin (siglecs), and dendritic cell-specific ICAM-3 grabbing nonintegrin (DC-SIGN). Ficolins were discovered much later than other members of the collectin family, and active investigation of their molecular structure and function as well as their clinical relevance is currently in progress.

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Discovery of Ficolins

Ficolin was originally discovered in early 1990 as a protein that binds to transforming growth factor-β (TGF-β). However, it now seems that ficolin was binding to the activated sepharose resin that was used for purification. This conclusion is also supported by the fact that native and recombinant ficolin failed to bind to TGF-β. The originally discovered porcine protein led to the cloning of two related genes that consist primarily of a FBG-like domain and a collagen-like domain; hence the genes were named ficolin-α and ficolin-β (Ichijo *et al*., 1993). Shortly thereafter, three homologous proteins were identified in humans, M-ficolin, L-ficolin and H-ficolin, which are also called ficolin-1, -2 and -3, respectively.

H-ficolin was initially discovered as a thermolabile β_2-macroglycoprotein, namely, Hakata antigen, which was originally found to be an autoantigen in systemic lupus erythematosus (SLE) patients. It was named after the city where the index patients were identified and later renamed to H-ficolin when complementary deoxyribonucleic acid (cDNA) of the antigen was cloned and found to be homologous to the ficolins. L-ficolin was identified and labelled P35 (protein size 35 kD) serum lectin, and

separately discovered as a corticosteroid-binding protein, labelled hucolin, by two independent research groups. M-ficolin was also discovered twice independently and named P35-related (for its homology to L-ficolin P35) as well as elastin-binding protein 37 (EBP37), as it binds elastin and is 37 kD in size.

Gene and Protein of Ficolins

Genes encoding L- and H-ficolins are located on chromosome 9q34, whereas M-ficolin is located on chromosome 1p35. The complement component 5 gene is also located in the 9q34 region and many other complement components and related proteins are found on chromosome 1 in humans. While humans have three ficolins, there are only two genes in the mouse, ficolin-A and ficolin-B, which are homologous to L- and M-ficolin, respectively. In the mouse and rat, a gene homologous to H-ficolin is a pseudogene due to the introduction of a stop codon due to base pair deletion in an exon. In nonmammalian vertebrates, *Xenopus* has four proteins homologous to ficolins. In nonvertebrates, the solitary ascidian (*Halocynthia roretzi*), a primitive marine animal (sea squirt), also has four homologous proteins.

The primary protein structure consists of three major domains: (1) collagen-like domain; (2) neck region and (3) FBG-like domain at the *C*-terminus (**Figure 1**). Delineation of the neck region is based on amino acid sequence alignment. The exon–intron organization of the FBG-like domain is well conserved among three ficolins. Single ficolin chains associate to form homotrimers, which serve as the functional subunit.

Ficolin trimers further associate to form multimers of the trimeric subunits. Multimerization of the trimers requires the presence of a second cysteine residue in the GXY repeat in the collagen domains, unlike collectins, which require the neck region for oligomerization in addition to cysteine residues located in the *N*-terminus of the collagen domain. H-ficolin forms hexamers, consisting of dimers of trimeric subunits (18 single peptides), whereas L-ficolin multimerizes to as high as octamers, which consist of dimers of tetramers (24 single peptides; **Figure 2**).

The structural organization of ficolins resembles complement component C1q and mannose-binding lectin (MBL) (Bohlson *et al.*, 2007). Although ficolins do not share any linear sequence homology with C1q, they are homologous to the collagen domain of MBLs. The most functionally relevant homology between ficolin and MBL is that both have a conserved region that is a putative MBL-associated serine protease (MASP) binding site, as discussed later.

The ficolin collagen domain is homologous to that of MBLs and nonfibrous collagen, suggesting that the collagen domains of MBLs and ficolins originated from a common precursor nonfibrous collagen gene. The FBG-like domain of ficolins is 40–50% homologous to that of tachylectin, a coagulation protein in horseshoe crab haemolymph, suggesting that the FBG-like domain might be derived from the clotting factor, fibrinogen (Krarup *et al.*, 2004). Based on these findings, it is suggested that ficolin, a chimeric protein of collagen and fibrinogen, might have arisen through gene shuffling.

Tissue Distribution of Ficolins

The tissue distribution of ficolin genes and protein is summarized in **Table 1**.

In humans, H-ficolin is predominantly synthesized in the lung and circulates in serum (Matsushita *et al.*, 1996). H-ficolin protein is also localized on epithelial cells in the lung and bile ducts, suggesting roles of this protein in respiratory and digestive functions in addition to its systemic function. Interestingly, the homologue to H-ficolin is missing in mouse and rat, as a result of the silenced gene, described earlier.

L-ficolin also circulates in serum, although it is primarily synthesized in liver (Matsushita *et al.*, 1996). The murine homologue, ficolin-A, is also expressed in liver from embryonic stages throughout adulthood, although in the spleen it is expressed minimally in the early neonatal period, increasing to strong expression from the late neonatal stage to adulthood. On detailed examination, ficolin-A is localized to macrophages in the liver and spleen as well as to lymphocytes in the spleen.

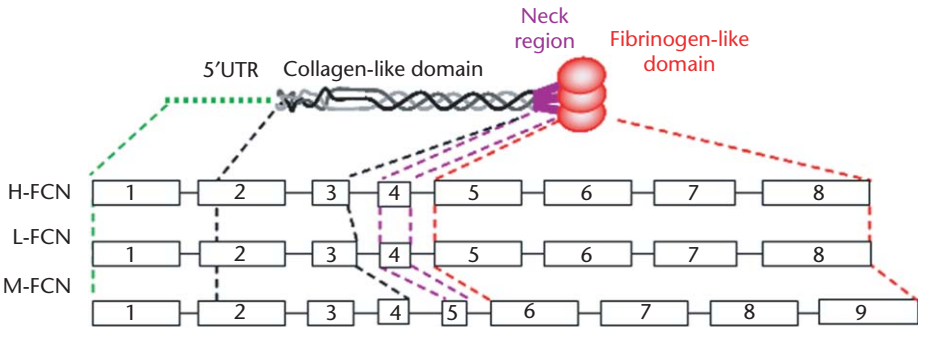

Figure 1 Gene and protein structure of human ficolins. The exon organization of ficolins is shown in relation to the domain structure of a single functional subunit, a trimer of ficolins. The size and distance of the exons and genes do not reflect actual sequence length.

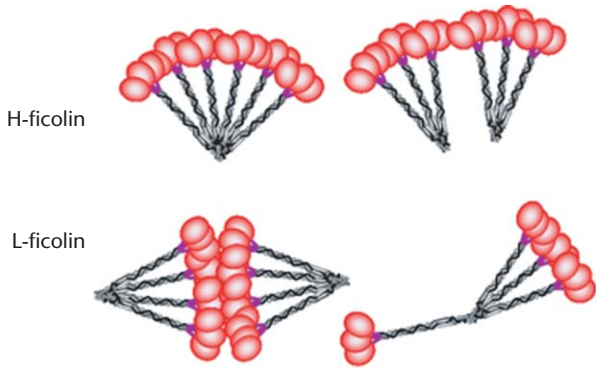

Figure 2 Molecular organization of ficolins. Schematic oligomers are depicted.

Table 1 Tissue distribution of human ficolins

Ficolins	Gene expression	Protein localization
L-ficolin	**Liver**	Plasma
	Lung	
	Spleen	
M-ficolin	**Peripheral blood leucocytes**	Macrophages
	Spleen	Granules of neutrophils
	Lung	
	Skeletal muscle	
	Bone marrow	
H-ficolin	**Lung**	Ciliated bronchial pneumocytes
	Liver	Type II pneumocytes
		Plasma
		Hepatocytes
		Bile duct epithelial cells

Note: Tissues in bold are the primary sites of ficolin messenger ribonucleic acid (mRNA) expression.

Unlike the other two human ficolins, M-ficolin is predominantly expressed by peripheral blood leucocytes and although it is secreted, it has not been detected in circulation. Similar to human M-ficolin, the murine homologue ficolin-B is expressed on leucocytes, which have been further characterized as neutrophils. Additionally, B-ficolin is also localized to the lysozyme of activated macrophages. Ficolin-B is strongly expressed in embryonic liver during the peak of organogenesis, decreases markedly towards the late neonatal stage and becomes undetectable in adult liver. It is therefore intriguing that the FBG domain of ficolin has similarity to tenascin, which is thought to be involved with tissue organization during embryonic development.

In any case, the pattern of tissue distribution and expression may suggest that each ficolin may have different roles under different circumstances, including at different points in development, as well as different targets.

Ficolins as Pattern-recognition Molecules

Ficolins are pattern-recognition molecules of the innate immune system, similar to the collectins (21197) which selectively recognize and bind to carbohydrates that decorate infectious agents and damaged self cells, such as apoptotic and necrotic cells (**Table 2**). Ficolins are able to selectively bind to compounds such as lipopolysaccharide (LPS) and lipoteicoic acid (LTA), among others, which are found on pathogens such as *Salmonella typhimurium*, *Staphylococcus aureus* and Group B streptococcus. Infection with these bacteria may cause illness in the infected host, and in humans may result in clinical complications and fatality, even despite treatment in many cases.

Ficolin binding to pathogens increases phagocytosis and initiates complement activation through the lectin pathway, resulting in elimination of the pathogen, as discussed later. However, the mechanisms by which ficolins recognize some pathogens remain unclear. In some cases, ficolin binding is not inhibited by carbohydrate and is not calcium dependent.

In terms of ligand selectivity, ficolins favour sialic acid and acetylated compounds, of which *N*-acetylglucosamine (GlcNAc) and *N*-acetylmannosamine are also recognized by collectins. Sialic acid has been thought to be a marker of self as it is the penultimate and the terminal sugar that usually decorates mammalian glycoproteins. Collectins, which function in part to distinguish self from nonself, do not bind sialic acid. L-ficolin seems to be the only mammalian serum lectin that can recognize a β-1,3-D-glucan which decorates fungi, such as yeast. This ligand selectivity suggests that ficolins may have different functions from collectins while at the same time sharing some similar functions. For example, MBL, L-ficolin and H-ficolin bind to apoptotic cells; however, H-ficolin does not bind to DNA unlike MBL and L-ficolin, suggesting that H-ficolin recognizes different molecule(s) on these dying cells. In addition, L-ficolin binding to apoptotic cells is not inhibited by GlcNAc, which is thought to be a likely ligand. Another class of sialic acid-binding lectin, siglecs, has been found to cause cell death upon binding to sialic acid on myeloid cells. Taken together, these findings suggest that some ficolins may share the capacity, along with siglecs, to induce cell death to eliminate cells of the immune system that are senescent or quiescent and are no longer needed for immunity.

The three-dimensional structure of the FBG domain in the presence and absence of a ligand has been determined for all human ficolins. The FBG domain forms a homotrimer, and each monomer has one calcium-binding site, as expected from previous studies. The calcium-binding site is homologous to that of tachylectin 5, one of the lectins isolated from

Table 2 Binding targets of ficolins

	Targets of ficolins		
Ficolins	Pathogens[a]	Endogenous	Ligands
L-ficolin	Group B streptococcus *S. pyogenes* *B. subtilis* *B. animalis* *S. typhimurium* *S. aureus* LTA[c]	Apoptotic cells Necrotic cells DNA	Acetylated compounds[b] β-1,3-D-glucan Sialic acid
M-ficolin		Apoptotic cells Acetyl-HSA[d]	Acetylated compounds Sialic acid
H-ficolin	*A. viridans* LPS[e]	Apoptotic cells Necrotic cells	Acetylated compounds D-fucose Galactose

[a]*S. pyogenes*, *Streptococcus pyogenes*; *B. subtilis, Bacillus subtilis*; *B. animalis, Bifidobacterium animalis*; *S. typhimurium*, *Salmonella typhimurium* and *A. viridans*, *Aerococcus viridans*.
[b]Acetylated compounds include *N*-acetylglucosamine, *N*-acetylgalactosamine, *N*-acetylneuraminic acid, *N*-acetylmannosamine, *N*-acetylglycine, *N*-acetylcysteine, acetylcholine and acetylated low-density lipoprotein (LDL).
[c]LTA, lipoteicoic acid.
[d]HSA, human serum albumin.
[e]LPS, lipopolysaccharide.

horseshoe crab haemolymph. The calcium-binding site is required to stabilize ligand binding, similar to other collectins. The CRD of surfactant protein A, a collectin, has only one calcium-binding site while MBL and surfactant protein D, also collectins, have three sites. This difference in calcium binding may explain the finding that ficolins can bind to some of their ligands independent of calcium, whereas MBL requires calcium for all ligand binding.

L-ficolin has four ligand-binding sites, unlike other ficolins and collectins that have only one site. The multiple binding sites allow L-ficolin to accommodate a broader ligand selectivity, including a linear β-1,3-D-glucan, of which a three-glucose repeat is found to fit in the binding pocket. Another ficolin homologue, the clotting factor G from horseshoe crab haemolymph binds to β-1,3-D-glucan and initiate clotting.

High multimerization states are known to be important for MBL to bind microbial surfaces, which carry ligands that span 45–50 Å. However, this model may apply differently for L-ficolin if each monomer accommodates a sugar chain, as discussed earlier. It has been found that a smaller oligomer, a tetramer (4 homotrimeric subunits), binds more strongly to GlcNAc than the octamer form (dimers of tetramers). Ficolins may also recognize pathogens through strong affinity to other ligands, including acetylated carbohydrates, LTA and D-fucose that are expressed on Gram-positive organisms and certain other pathogens.

The Role of Ficolins in Innate Immunity and Human Disease

Two serum ficolins, H-ficolin and L-ficolin, have a wide range of normal plasma concentration, 2–40 $\mu g\,ml^{-1}$ and 1–30 $\mu g\,ml^{-1}$, respectively. These ranges are in part attributable to multiple single nuclear polymorphisms (SNPs) throughout the gene, as summarized in **Figure 3**. At this time, the relationship between SNPs and serum concentration has been best evaluated for L-ficolin. It has been found that amino acid substituting SNPs in the FBG coding region, the binding domain, does not markedly reduce the serum concentration. However, amino acid substitution at position 258 (alanine to serine) results in reduced serum concentration, although binding to GlcNAc is increased. Within the promoter region, two SNPs increase serum concentration, while another is associated with reduction. Like L-ficolin, H-ficolin also has SNPs; however, none have been reported in association with H-ficolin deficiency. As discussed earlier, H-ficolin was originally identified as an autoantigen in SLE patients, whose serum levels are low, due to increased circulating antibodies to H-ficolin. H-ficolin blood levels have been correlated with severity of liver cirrhosis, with decreasing blood levels associated with more severe disease, although the liver is not the primary site of H-ficolin synthesis. The numbers of SNPs correlated with ficolin phenotypes may increase, as genetic analysis of ficolins has begun only recently compared with collectins.

As ficolins are functionally and structurally similar to collectins, and since many clinical studies have found that collectin deficiency due to SNPs is associated with increased susceptibility to infection (although there are contradictory reports as well), there has been considerable speculation about the significance of ficolins in human disease. A few clinical studies have failed to find a correlation between L-ficolin deficiency and infection susceptibility in adult patients with chemotherapy-induced neutropenia and with invasive pneumococcal disease. In contrast, low

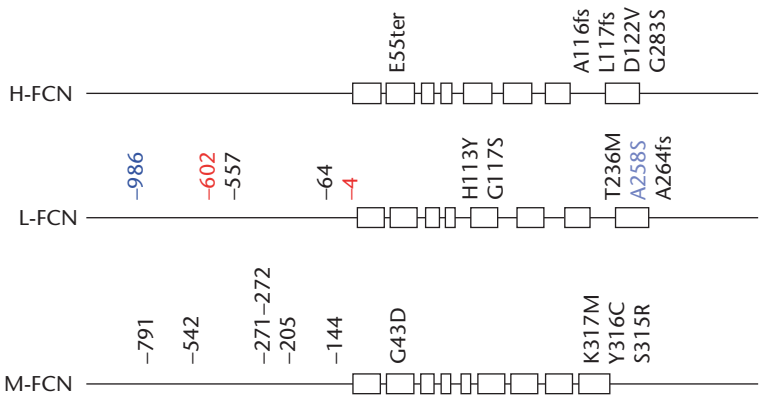

Figure 3 Gene polymorphisms in ficolin genes. SNPs in gene sequence numbers and amino acid substitution polymorphisms in protein sequence numbers are indicated. SNPs in black, blue and red indicate no change, reduction and increase of blood levels, respectively. Sizes of promoter, exon and intron do not reflect actual distance.

serum concentration of L-ficolin has been found in association with recurrent respiratory infection in children (Atkinson *et al*., 2004). Low blood levels of L-ficolin have been associated with pregnancy-related problems, such as miscarriage and preeclampsia (Wang *et al*., 2007). In the latter case, H-ficolin was also found to be low, and in contrast, both H- and L-ficolins were abundant in placental extract and have been localized by immunohistochemistry to apoptotic syncytiotrophoblasts. In the same study, increased ficolin levels were positively correlated with an increase in certain chemokines and negatively correlated with other cytokines in the placenta, suggesting a modulatory role for ficolins in inflammation.

One of the functions of ficolins is the activation of the complement cascade by the lectin pathway, which can also be activated by MBL. The lectin pathway is initiated in cooperation with MBL-associated serine protease (MASP). Four MASPs, MASP-1, -2, -3 and sMAP (Map19) exist and are known to form complexes with ficolins and MBLs in circulation (Fujita *et al*., 2004). MBLs and all ficolins have putative MASP-binding sites in the collagen domain (Girija *et al*., 2007). Ficolin ligand binding is required for the initiation of lectin pathway (**Figure 4**). Binding of L-ficolin and M-ficolin to bacterial polysaccharide and acetyl-HAS, respectively, has been shown to activate the lectin pathway. It has recently been found that ficolin may enhance or is required for bacterial killing mediated by C-reactive protein (CRP). Although CRP was once thought to kill bacteria by itself, a recent study suggests that CRP does not bind nor activate complement by itself.

A murine knockout of ficolin A (homologue of L-ficolin) has been produced and is currently being used for investigation of disease models. Although the animal model study may not be powerful tool because (1) ficolin and MBL are similar in their selectivity for ligands, such as GlcNAc and LTA, and in their ability to activate the lectin complement pathway and (2) there are only some $\mu g\,ml^{-1}$ of ficolin A, compared with a total of approximately

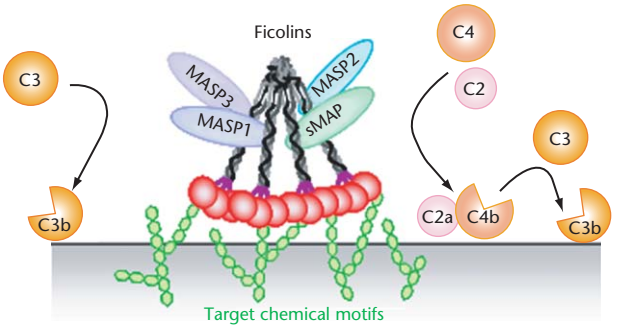

Figure 4 Activation of complement by ficolin–MASP complex.

$60\,\mu g\,ml^{-1}$ of MBL-A and MBL-C. Given these findings, it may be that MBL may represent the primary innate immune lectin in mice. Nevertheless, these things remain to be tested, and animal model studies may yet prove to be useful for elucidating the roles of ficolins *in vivo*.

Conclusion

The innate immune system provides instant protection from invading pathogens as well as helping to maintain the healthy state through clearance of dying cells and their debris. The scientific and clinical community is paying increased attention to ficolins for their role in innate immunity. The similarity of ficolins to MBL may serve as a useful model for understanding their function, in that MBL is an important pattern-recognition molecule of the innate immune system in addition to its role as a modulator of inflammation. In humans, a significant difference from MBL is that ficolin has three homologues, H-, L- and M-ficolin. These three ficolins share some similar functions but at the same time the three have different tissue distributions and different target selectivity, suggesting that each may have a unique function in diverse processes, including innate immunity, tissue homeostasis and development. Like

collectins, ficolins also seem to cooperate with other molecules of the innate immune system, adding additional complexity and routes of modulation and function in host defense. The pace of scientific and clinical investigations is increasing, in the search for understanding of the function of ficolins in the innate immune system in health and disease.

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