A peer reviewed journal with structured data

The Molecule Pages from UCSD Signaling Gateway have been regularly published online since one decade and are now printed biannually. As a researcher involved in biomathematical and biomechanical modeling and simulation, I found these pages very informative, either when dealing with biological processes happening at the nano- and microscopic scales, or incorporating these events in a meso- and macroscopic scale modeling to enhance reductionist models when necessary. UCSD Molecule Pages indeed yield information that enables interdisciplinary research and I have cited the Molecule Pages over 130 times in my recent book ‘Intracellular Signaling Mediators in the Circulatory and Ventilatory Systems’ (Springer New York, 2013).

Any paper published in a journal specialized in molecular biology focuses on specific aspects of the signaling protein in a given context. On the other hand, any UCSD Molecule Page provides a full description of the protein and its structure-function relationship. Associated historical section explains different names, old ones being still used by some authors nowadays. A major aspect, which a non-specialist generally confronts, is the large number of names assigned to a single protein. Furthermore, a given protein alias can define many different types of proteins. In addition, the meaning of a protein alias is often very difficult to obtain in the literature. UCSD Molecule Pages should be always targeted for such disambiguation.

Each Molecule Page describes a cell signaling mediator. It starts with a summary with alternative names and aliases as well as a network map. The latter is accessible directly from the left menu. The full text contains a series of items that include the protein structure and function, regulation of activity, binding partners, regulation of concentration in physiological and pathological conditions, subcellular localization, major sites of expression, phenotypes, alternatively spliced variants, and antibodies to end with a list of references. Therefore, UCSD Molecule Pages are indispensable tools for any biologist and bioinformatician who wants to have an overview on the state of the art and find a precise molecule feature. They can be used with success by any researcher working in other scientific fields with applications to biology to get an overall knowledge of a given molecule.

UCSD Molecule Pages that are beneficial for a large fraction of the entire community of researchers should be maintained and regularly updated. The Molecule Pages which will be published in this issue are: p38 beta MAP kinase (A001718), Mannose/mannan-binding lectin (A004276), Leukocyte antigen CD47 (A005186), L-ficolin (A004266) and TSH receptor (A002333).

-Marc Thiriet
MOLECULE PAGES

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p38 beta MAP kinase

Simon Rousseau

In mammals, there are four p38 protein kinases: p38α, p38β, p38γ and p38δ. p38β was identified in 1996 as a closely related protein kinase of p38α, sharing 74% sequence identity and the Thr-Gly-Tyr dual phosphorylation motif characteristic of all p38 MAPKs. p38β is widely distributed in cells and tissues, but less so than p38α; p38β is particularly abundant in endothelial cells. p38β is activated in vivo by dual phosphorylation at Thr180 and Tyr182 by the MAP2K, MKK3 and MKK6 in response to a multitude of stimuli including environmental stressors, cytokines and growth factors. p38β can be dephosphorylated on both its Thr and Tyr residues by Dual-Specificity Phosphatases. p38β, like p38α, is targeted by a class of pyridinyl imidazole drugs that do not target the other two p38 MAPKs. These compounds were invaluable in discovering functions regulated by p38α and p38β. However, they do not permit to distinguish functions mediated by p38β from those regulated by p38α. This distinction has been made possible by the use of genetically engineered mice. p38β-deficient mice are not embryonic lethal such as those lacking p38α. However ectopic expression of p38β can rescue the lethality of p38α-deficiency. This suggests that p38α is the “dominant” form but that functional redundancy exists between the two related protein kinase. p38β has been shown to play specific roles in gene expression, regulation of cell death, cell differentiation and neuropathic pain. However, p38β is not involved in transducing pro-inflammatory signals, myogenesis or cell motility, when p38α is present.

KEYWORDS

Mapk11; Mitogen-activated protein kinase 11; p38 beta MAP kinase; p38 beta Map kinase; p38 beta MAPK; p38-2; p38B; p38beta; p38beta MAPK; P38BETA2; Prkm11; Protein kinase, mitogen activated kinase, 11; SAPK2; SAPK2B

IDENTIFIERS

Molecule Page ID:A001718, Species:Mouse, NCBI Gene ID: 19094, Protein Accession:NP_035291.4, Gene Symbol:Mapk11

PROTEIN FUNCTION

Background

p38β was identified in 1996 as a closely related protein kinase of p38α, sharing 74% sequence identity and the Thr-Gly-Tyr dual phosphorylation motif characteristic of all p38 MAPKs (Jiang et al. 1996). p38β differs most significantly from p38α in the region located between kinase domains V and VI, creating two gaps totaling eight amino acids (Jiang et al. 1996). A comparison between the structures of the two protein kinases showed a difference in the orientation of the N- and C-terminal domains causing a reduction in the size of the ATP-binding pocket in p38β (Patel et al. 2009). p38β, like p38α, is targeted by a class of pyridinyl imidazole drugs that do not target p38γ or p38δ. These compounds were invaluable in discovering functions regulated by p38α and p38β. However, they do not permit to distinguish functions mediated by p38β from those regulated by p38α. Despite efforts to design compounds that target only one of the two-related protein kinases (Patel et al. 2009), this distinction has been made possible by the use of genetically engineered mice. p38β-deficient mice are not embryonic lethal such as those lacking p38α (Adams et al. 2000, Mudgett et al. 2000, Beadmore et al. 2005). However ectopic expression of p38β can rescue the lethality of p38α-deficiency (Okada et al. 2007). This suggests that p38α is the “dominant” form, but that functional redundancy exists between the two related protein kinases. In order to avoid major duplications between the p38α and p38β molecule pages, only functions shown to be mediated by p38β specifically are reported in the next sections. However, the reader is referred to the p38α molecule pages to obtain a more complete overview of the functions regulated by these two protein kinases.

Transcriptional regulation

p38β phosphorylates ATF2 and increases its transcriptional activity (Jiang et al. 1996, Lee et al. 2002); this can be decreased through its interaction with histone deacetylase 3 (HDAC3) (Mahlknecht et al. 2004). p38β increases AP-1 transcriptional activity induced by arsenite in human breast cancer cells (Pramanik et al. 2003). p38β also phosphorylates ATF7 at Thr51, which prevents ATF7 sumoylation, enabling the interaction of ATF7 with TAF12, thus increasing transcription (Camuzeaux et al. 2008).

Cell cycle and cell death

Cell cycle Regulation

Carbon monoxide (CO)-induced activation of p38β up-regulates caveolin-1, which inhibits smooth muscle cell proliferation (Kim et al. 2005a). Additionally, IFNα regulates growth inhibition of Jurkat cells through p38α and p38β (Lee et al. 2010).

Stimulation of cell death

p38β is required for anoikis in undifferentiated intestinal epithelial cells (Vachon et al. 2002). Moreover, cardiomyocyte apoptosis induced by a dominant negative 14-3-3η targeted to postnatal cardiac tissue is mediated mostly by p38β, with a lesser contribution from p38α (Zhang et al. 2003). Furthermore, cardiomyocyte apoptosis induced by expression of Related Adhesion Focal Tyrosine Kinase (RAFTK) requires p38β (Melendez et al. 2004).

Protection from cell death

As is the case for p38α, p38β has also been implicated in the
protection from cell death. CO cytotoxic effects against oxidative stress in endothelial cells occur via p38β regulation of heat shock protein 70 (Otterbein et al. 2003, Kim et al. 2005b). Moreover, isoflavone Genistein induces p38β activity, which protects endothelial cells from TNFα-induced apoptosis (Si and Liu, 2009).

Cell differentiation

During keratinocyte differentiation, down regulation of the transcription factor E2F1, requires p38β (Ivanova et al. 2006). p38β contributes to C2C12 myogenic differentiation in conjunction with with p38α and p38γ, by selectivity regulating cyclin D3, a unique target of p38β (Wang et al. 2008). Moreover, osteoclast differentiation requires p38β activation by TAK1 (Greenblatt et al. 2010).

Metabolism

p38β was found to phosphorylate glycogen synthase (GS) at Ser644, which enables subsequent phosphorylation of GS by Glycogen Synthase kinase 3 (GSK3) resulting in inhibition of GS activity (Kuma et al. 2004).

Nervous system

p38β binds and phosphorylates the neurotrophin receptor p75(NTR) which results in enhanced NFκB activity and decreases AP-1 activity in Schwann cells (Wang et al. 2000). The microtubule associated protein Tau is a good substrate of p38β, which can phosphorylate Thr181, Ser202, Thr205, Ser396, Ser404 and Ser422 (Buee-Scherrer and Goedert 2002).

Neuropathic pain

p38β expressed in the microglia of the spine and plays a role in spinal nociceptive processing (Svensson et al. 2005). Spinal hyperalgesia was prevented by down regulation of p38β but not p38α using antisense oligonucleotides (Fitzsimmons et al. 2000). The microtubule associated protein Tau is a good substrate of p38β, which can phosphorylate Thr181, Ser202, Thr205, Ser396, Ser404 and Ser422 (Buee-Scherrer and Goedert 2002).

Cardiovascular system

Shear-stress mediated expression of chemokines is mediated by p38β in endothelial cells (Shaik et al. 2009).

Functions shown to be mediated by p38α and not p38β

Mice-lacking p38β have a slight reduction in MAPKAP-K2 activity and no reduction in MSK1 activity in response to anisomycin, demonstrating that p38α is the main isoform responsible for MAPKAP-K2 and MSK1 activation (Beardmore et al. 2005). Accordingly, p38α is the main form involved in mediating cytokine production, as mice lacking p38β show no defect in cytokine production or immune functions (Beardmore et al. 2005). Similar results were obtained through chemical genetics analysis of p38α and p38β inhibition (O’Keefe et al. 2007). p38α is the essential p38 isoform sustaining adult myogenesis (Ruiz-Bonilla et al. 2008). In cells derived from mice lacking the four different p38 MAPKs, it was found that only p38α was involved in relaying chemotactic signals (Rousseau et al. 2006). The activation of p38α but not p38β is required for ischemic preconditioning of the heart (Sicard et al. 2010).

REGULATION OF ACTIVITY

Dual phosphorylation by MKKs

The canonical activation of p38β occurs via dual phosphorylation of the pThr180-Gly181-pTyr182 motif, in the activation loop by MKK6 or MKK3, but not MKK3 splice variants missing the N-terminal 29 amino acids (i.e. lacking its docking site) in contrast to p38α (Jiang et al. 1996, Enslen et al. 2000). Therefore, the presence of a docking site is necessary for p38β activation by MKKs (Enslen et al. 2000). Thr180 and Tyr182 are exposed to the surrounding solvent on the activation loop, and in the absence of phosphorylation interfere with substrates binding (Bellon et al. 1999). Upon phosphorylation, p38β goes through characteristic global conformational changes that alter the alignment of the two kinase halves (N-terminal and C-terminal domains) and enhance access to substrate, which increases enzymatic activity (Bellon et al. 1999, Canagarajah et al. 1997).

Dephosphorylation

The magnitude and duration of p38β signal transduction are critical determinants of its biological effects. Therefore p38β inactivation is a crucial part of the biological responses it controls. It is believed that the same phosphatases acting on p38α are responsible for inactivating p38β. These include the members of the PP2C family, (Takekawa et al. 1998, Takekawa et al. 2000) and DUAL-Specificity MAPK kinase Phosphatases (DUSP; also known as MAPK Kinase Phosphatases, MKP) (Dickinson and Keyse 2006). DUSP8 (also known as M3/6) was the first phosphatase shown to specifically target stress-activated protein kinases (Muda et al. 1996). The inducible nuclear DUSP, DUSP1 (also known as MKP-1) also dephosphorylates p38β (Franklin and Kraft 1997, Chu et al. 1996, Dickinson and Keyse 2006).

INTERACTIONS

p38β was found to interact with the neurotrophin receptor p75(NTR) (Wang et al. 2000). p38β was found to interact with the MAPK docking site (D-site) in the N-terminus of MKK4 (Ho et al. 2003) and the protein kinase MAPKAP-K5 (also known as PRAK) (New et al. 2003). p38β binds the phosphatase DUSP16 (Tanoue et al. 2001). Unphosphorylated p38β interacts with the N-terminus of HDAC3 (Mahlknecht et al. 2004). p38β, but not p38α, interacts with Glycogen Synthase in skeletal muscle, liver and brain (Kuma et al. 2004).

PHENOTYPES

p38β-deficient mice do not die due to placental defects like p38α-deficient ones (Adams et al., 2000, Mudgett et al., 2000); they are born viable and fertile (Beardmore et al., 2005). p38β-deficient fibroblasts did not induce caveolin-1 in response to CO, which was restored by p38β gene transfer (Kim et al., 2005). In mice lacking p38β, p38γ and p38δ, the regeneration and myofiber development of adult muscle proceeded as in wild type mice, excluding a role for p38 MAPKs other than p38α in mediating muscle growth (Ruiz-Bonilla et al., 2008). However, mice lacking p38β were found to have reduced bone mass secondary to defective osteoclast differentiation (Greenblatt et al., 2010). In contrast to the other members of the p38 MAPK family, the dephosphorylation of S6K1 induced by 2-deoxy-glucose is prevented in p38β-deficient mouse embryonic fibroblasts (Zheng et al., 2011). This suggests a role for p38β in stress induced inhibition of cell growth.

MAJOR SITES OF EXPRESSION

p38β is widely distributed in cells and tissues, but less than p38α (Beardmore et al. 2005). Northern hybridization showed expression in human brain, heart, placenta, lung, liver, skeletal
muscle, kidney, and pancreas (Jiang et al. 1996). p38β has not been detected in monocytes, macrophages, neutrophils. However, low amounts of p38β were shown to be present in CD4+ T cells and abundantly in endothelial cells (Hale et al. 1999). In rheumatoid arthritis patients, p38β expression was found in synovial fibroblasts and endothelial cells, whereas the dominant p38 MAPKs found in inflamed tissue were p38α and p38γ (Korb et al. 2006). In the spinal dorsal horn, p38β is expressed in the microglia in contrast to p38α, which is expressed in neurons (Svensson et al. 2005). In the mouse brain, p38β was found to be expressed in the nucleus of neurons in contrast to p38α, which was found mostly in dendrites, cytoplasmic and nuclear regions (Lee et al. 2000). In the post-ischemic brain, p38β activity was biphasic; with early increase in the nuclei and dendrites of neurons and the late activation in astrocytes found in the penumbra (Piao et al. 2003).

**SPLICE VARIANTS**
No splice variants of p38β have been reported.

**REGULATION OF CONCENTRATION**
Diazoxide, a potassium channel activator, up-regulates p38β expression by pancreatic β-cells (Huang et al. 2007). p38β expression has been shown to be up-regulated in the progression of malignant astrocytoma cells (Zeng et al. 2008).

**ANTIBODIES**
To detect specifically all forms of p38β (phosphorylated and non-phosphorylated), we have previously used the R&D systems anti-p38β (cat # MAB5885). To detect phosphorylated p38β only, all p38β forms can be immuno-precipitated first with the aforementioned antibody and the phosphorylation state detected with a pan-pThr180-pTyr182 antibody, such as the Millipore anti-phospho-p38 (pThr 180/Tyr 182; cat no 09-272).
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REFERENCES


This molecule exists in 18 states, has 18 transitions between these states and has 6 enzyme functions. (Please zoom in the pdf file to view details.)
Mannose/mannan-binding lectin

Ashok Reddy Dinasarapu¹, Anjana Chandrasekhar¹, Teizo Fujita², Shankar Subramaniam¹

Mannose/mannan-binding lectin (MBL) is a serum lectin synthesized (as a ~32 kDa peptide) by the liver and is one of the key molecules of the innate immune system. Each peptide has an N (amino)-terminal cysteine-rich region, a middle stretch of a collagen-like sequence, and a carbohydrate recognition domain (CRD) in the C (carboxy)-terminus. Three identical peptides form a structural subunit, similar to a collagenous triple helix, which is the basic building block of all circulating molecular forms of MBL. Further oligomerization of these structural subunits by disulphide bonds in the N-terminal region results in MBL molecules of different sizes (from dimers to hexamers), but the hexameric form is probably the most common. MBL-associated serine proteases (MASPs) bind to MBL multimeric forms to stabilize the molecule. MBL is a pattern-recognition receptor and the CRD of MBL serve to bind to a wide range of pathogens such as bacteria, viruses and protozoa, by recognizing carbohydrate moieties on their surfaces. There are two pathways by which MBL can participate in a host defense response: 1) MBL activates the lectin complement pathway via MASPs, that converges with the classical complement pathway, at the level of complement C4 (C4-A or C4-B), and 2) MBL may also act directly as an opsonin, enhancing phagocytosis by binding to cell-surface receptors present on phagocytic cells.

KEYWORDS
COLEC1; Collectin-1; HSMBPC; Mannan-binding lectin; Mannan-binding protein; Mannose-binding lectin; Mannose-binding lectin (protein C) 2, soluble; Mannose-binding lectin protein C 2, soluble (opsonic defect); Mannose-binding lectin 2, soluble (opsonic defect); Mannose-binding lectin protein C; Mannose/mannan-binding lectin; MBL; MBL2; MBL2D; MBP; MBP-C; MBP1

IDENTIFIERS
Molecule Page ID:A004276, Species:Human, NCBI Gene ID: 4153, Protein Accession:NP_000233.1, Gene Symbol:MBL2

PROTEIN FUNCTION
Mannose/mannan-binding lectin (MBL), previously known as mannann-binding protein (MBP) is a (C-type or calcium dependent) serum lectin with primary specificity for sugars such as D-mannose, N-acetylgalosamine (GlcNAc), N-acetylmannosamine (ManNac) and L-fucose (Kawasaki et al. 1983, Sheriff et al. 1994). It circulates in serum as tri- to hexameric forms of the structural subunit (see ‘Interactions with Ligands and Other Proteins’ section) in association with a group of MBL-associated serine proteases (MASPs) (Matsushita and Fujita 1992). Being part of the innate immune system, MBL recognizes pathogens and damaged cells, by binding to carbohydrate moieties on microorganisms and altered self-surfaces. MBL firmly bound to foreign or altered self-surfaces, can participate in host defense response by activation of: the lectin complement pathway, phagocytosis, apoptotic cell clearance and inflammatory processes. As MBL is structurally similar to C1q, MBL can also compete with C1q for binding to altered self-ligands (Oroszlán et al. 2007, Agostini et al. 2012).

Complement activation: The lectin pathway of complement activation, initiated through MBL-MASP or Ficolin-MASP cascades, is antibody- and C1q-independent. MBL binds to specific carbohydrate structures found on the surface of a range of microorganisms in association with MASPs and activates the complement system (Ji et al. 1993, Thiel et al. 1997, Kawasaki et al. 1989). On binding to appropriate targets, the MASP-1 activated MASP-2 (both the MASPs are in the MBL-MASP complex) sequentially cleaves complement factors C4 and C2 leading to the formation of C3-convertase (C4b2a) (Thiel et al. 1997). The C3-convertase is a complement C3 specific enzyme which cleaves C3, into C3a and C3b fragments. In mice, MBL via MASP-1 and MASP-3 has been shown to be essential for activation of complement factor D and the alternative complement pathway (Iwaki et al. 2011, Takahashi et al. 2010).

Opsonization and Phagocytosis: MBL can function directly as an opsonin by binding to pathogen, or indirectly by producing opsonins like C3b. These opsonized pathogens/particles are recognized by a number of putative binding proteins/phagocytic receptors including, calreticulin/CD91 (cC1qR/LRP-1) (Ogden et al. 2001, Malhotra et al. 1990), C1QR1 (C1qRp, CD93) (Tenner et al. 1995) and complement receptor type-1 (CR1, CD35) (Ghiran et al. 2000). Calreticulin, an endoplasmic reticulum (ER) protein that acts as a chaperone during protein assembly, can be recruited to the cell surface during phagocytic recognition (Gagnon et al. 2002). Low levels of serum MBL are associated with defects in C3b opsonization on yeast surfaces and recurrent infections in children, which imply a role for MBL in host defense in humans (Super et al. 1989, Turner et al. 1981). MBL (from MBL-coated Salmonella montevideo) was able to interact directly with cell surface receptors and promoted opsonophagocytosis (Kuhlman et al. 1989). MBL can opsonize Human immunodeficiency virus 1 (HIV-1) but does not induce neutralization at the levels at which it is normally present in serum. However, binding and opsonization of HIV by MBL may alter virus trafficking and viral antigen presentation during HIV infection (Ying et al. 2004). MBL and C1q (as MBL is structurally similar to C1q), modulate monocyte activation and chemokine responses during the clearance of oxidized (Ox) LDL. MBL has been reported to directly bind OxLDL and enzymatically modified forms of LDL (E-LDL) in OxLDL-loaded monocytes and human monocyte derived macrophages (HMDM) and can therefore enhance cholesterol efflux (Fraser and Tenner 2010).

Recognition and clearance of altered-self: Role for MBL in the
clearance of apoptotic cells was suggested through antibody blockade studies that showed that inhibition of calreticulin and CD91 blocked collectin mediated uptake of apoptotic cells by macrophages (Ogden et al. 2001). MBL was found to bind directly to apoptotic cells that expose terminal sugars of cytoskeletal proteins, thereby permitting their recognition and directly facilitating their phagocytosis by macrophages. However, it is important to note that MBL can also act as an opsonin by mediating uptake not only via collectin receptors but also through the generation of C3 opsonins (C3b and iC3b) that coats the targets and triggers uptake by complement receptor type 3 (CR3,CD18/CD11b) (Ip et al. 2009). Changes in cell surface structures during oncogenic transformation appear to promote binding of MBL to cancer cells (Hakomori 2001), wherein the protein can mediate cytotoxic effects including MBL-dependent cell mediated cytotoxicity (Ma et al. 1999, Nakagawa et al. 2003).

Modulation of inflammation: MBL plays an important role in modulating inflammation, by releasing cytokines and interleukins. MBL is involved in the binding of cryptococcal mannoprotein (MP2) to human peripheral blood mononuclear cells (PBMCs) and the release of tumor necrosis factor-α (TNF-α) (Chaka et al. 1997). Likewise, PBMCs from HIV infected patients when bound to MBL, increase cytokine production and viral replication (Huggelund et al. 2005). Monocytes secrete higher levels of TNF-α, interleukin-6 (IL6) and IL-1β, when infected with MBL-opsonised Neisseria meningitides (Jack et al. 2003) or Leishmania chagasi promastigotes (Santos et al. 2001), as compared to non-opsonized bacteria.

REGULATION OF ACTIVITY

Hepatocyte gene expression and plasma levels of MBL are stimulated by peroxisome proliferator-activated receptor - α (PPARα) and fenofibrate (used to reduce cholesterol levels in humans at risk of cardiovascular disease). This evidence links PPARα to regulation of innate immunity and complement activation in humans, and suggests a possible role of MBL in lipid metabolism (Rakhshandehroo et al. 2012). The salivary scavenger and agglutinin (SALSA, gp340), binds to both pathogen surface and MBL. This interaction (when SALSA is bound to the surface) activates the lectin pathway, while pathogen surface and MBL. This interaction (when SALSA is bound to the surface) activates the lectin pathway, while bound MBL can also act as an opsonin by mediating uptake not only via collectin receptors but also through the generation of C3 opsonins (C3b and iC3b) that coats the targets and triggers uptake by complement receptor type 3 (CR3,CD18/CD11b) (Ip et al. 2009). Changes in cell surface structures during oncogenic transformation appear to promote binding of MBL to cancer cells (Hakomori 2001), wherein the protein can mediate cytotoxic effects including MBL-dependent cell mediated cytotoxicity (Ma et al. 1999, Nakagawa et al. 2003).

MBL-MASP Complex: MBL, as an oligomer (tri- to hexameric), forms complexes with different MASP proteins as listed below. All the MASP proteins bind to MBL in homodimeric form (Chen and Wallis 2001, Teillet et al. 2008, Thielens et al. 2001). However, the stoichiometry of the different components in the MBL-MASP complex is highly variable. MBL binds to MASP via its collagen domain (Super et al. 1992, Kurata et al. 1993).

MASP-1 and MASP-2: MASP-1 and MASP-2 encoded by MASP1 and MASP2 genes respectively, are serine proteases (Matsushita et al. 2000, Skjoedt et al. 2011, Thiel et al. 1997), which when activated, sequentially cleave complement proteins C4 and C2. MASP-1 is auto-activated, when in complex with MBL bound to microbial carbohydrates. Activated MASP-1 then activates MBL bound MASP-2 (Seke et al. 2013, Héja et al. 2012a, Héja et al. 2012b). MASP-1 can cleave complement C3 (weakly as compared to C3-convertase), complement factor D in mice (Takahashi et al. 2010) and appears to cleave complement C2 (Matsushita et al. 2000).

MASP-3: MASP-3 is a splice variant of MASP1 gene. It has a serine protease domain, but has no known substrates. It is believed to compete with MASP-2 to bind to MBL and therefore down-regulate lectin pathway activation (Dahl et al. 2001). In mice however, MASP-3 has been shown to be important in alternative pathway of complement activation (Iwaki et al. 2011). Higher oligomeric structures of MBL (larger than trimeric forms) are shown to be in complex with all the three MASP5 (MASP-1, MASP-2 and MASP-3) at the same time (Dahl et al. 2001).

MAP44 and sMAP (Map19): Map44, expressed mainly in the heart, is yet another splice variant of MASP1 gene. It however does not have a serine protease domain. It competes with MASP-2 to bind to MBL and down-regulates lectin pathway activation (Skjoedt et al. 2010, Degn et al. 2009). sMAP, a splice variant of MASP2 gene, also lacks a serine protease domain and competes with MASP-2 to bind to MBL, thereby down-regulating lectin pathway activation (Stover et al. 1999, Takahashi et al. 1999). It was found to be in complex with MASP-1 and trimeric form of MBL (Tateishi et al. 2011).

MBL-Host Interactions: MBL has been shown to bind directly to apoptotic (and necrotic) cells, and facilitate clearance of these cells by phagocytosis (Nauta et al. 2003, Ogden et al. 2001). The bound MBL on apoptotic cells stimulate ingestion by phagocyte by binding to calreticulin (cC1qR), which in turn is bound to the endocytic receptor protein CD91 (LRP-1, α2-macroglobulin receptor) (Eggleton et al. 2001). The bound MBL can also bind to complement receptor type 1 (CR1, CD35) and C1qR (CD93) receptors present on phagocytes (Ghiran et al. 2000, Malhotra et al. 1995). Therefore MBL deficiency might lead to the accumulation of apoptotic cells, thereby predisposing the host to systemic autoimmunity. MBL pathway is activated upon interaction with αβ1
MBL is responsible for activating complement on endothelial cells following periods of oxidative stress. Oxidative stress increases endothelial cytokeratin-1 (CK1) expression (Collard et al. 2001) and CK1 represents a candidate molecule as a MBL ligand under conditions of cell stress and injury (Collard et al. 2001, Montalto et al. 2001). Aberrant glycosylation patterns, like Lewis A and Lewis B (Lea–Leb), expressed on glycoproteins CD26 and CD98 heavy chain of human tumor cell lines derived from colon adenocarcinoma and colorectal carcinoma respectively (Muto et al. 1999, Muto et al. 2001), have been identified as ligands for MBL (Kawasaki et al. 2009). Furthermore, MBL has been implicated in activating complement by binding to glycosylated immunoglobulin(Ig)G isoforms associated with rheumatoid arthritis (Tenner et al. 1995), polymeric forms of IgA (Hisano et al. 2001, Roos et al. 2001, Terai et al. 2006) and certain glycoforms of IgM (Arnold et al. 2005).

MBL-Pathogen Interactions: MBL binds multiple bacterial polysaccharides having terminal monosaccharides such as D-mannose, GlcNAc, ManNAc and L-fucose but not galactose and siatic acids (which are present on host cells) (Weis et al. 1992, Drickamer 1992). In fact, some pathogens use the strategy of producing polysaccharide capsule and sialylation of lipopolysaccharide structures to escape MBL binding (Jack et al. 2001, Krarup et al. 2005). MBL has been shown to bind to a wide range of bacteria, viruses, fungi and protozoa (Dommett et al. 2006). However, the binding of MBL to pathogens differs both between and within species (heterogeneous binding patterns) (Townsend et al. 2001, Neth et al. 2000).

Teichoic acid of Staphylococcus aureus, a cell surface glycopolymer containing GlcNAc residue, has been shown to be a functional ligand of MBL (Park et al. 2010). In addition to Staphyloccocal aureus, several bacterial species have been found to bind to MBL including: Actinomyces israelii, Bifidobacterium bifidum, Leptotrichia buccalis, Propionibacterium acnes (Townsend et al. 2001); Burkholderia cepacia, Pseudomonas aeruginosa (Davies et al. 2000); Chlamydia pneumoniae (Swanson et al. 1998); Klebsiella aerogenes, Haemophilus influenzae, Streptococcus pneumoniae, Escherichia coli, Haemophilus influenzae, Neisseria meningitidis, Listeria monocytogenes, (van Emmerik et al. 1994, Neth et al. 2000); Mycobacterium avium (Polotsky et al. 1997); Mycoplasma pneumoniae (Hamvas et al. 2005); and Salmonella montevideo (Kuhlman et al. 1989).

Interestingly, other bacteria (anaerobic) that are most commonly implicated in clinical disease such as, Bacteroides and Clostridium, bound little or no MBL. Similarly, the only Veillonella species that causes any appreciable disease, V. parvula, bound little or no MBL. In contrast, Veillonellopsis dispar, Bifidobacterium bifidum, Propionibacterium acnes, Leptotrichia buccalis, which very rarely cause significant infections, bound to MBL. Also, Fusobacterium, a rarely isolated organism is bound to measurable amounts of MBL. This suggests that there may be an inverse relationship between pathogenicity and the level of MBL binding (Townsend et al. 2001). MBL can bind to viruses such as, influenza A, HIV (Hartshorn et al. 1993, Safiuddin et al. 2000, Hart et al. 2002, Ji et al. 2005), and severe acute respiratory syndrome (SARS) coronavirus (CoV) (Ip et al. 2005). A number of clinical studies have suggested that deficiency of MBL is a risk factor for acquiring HIV infection. MBL can bind to purified HIV-gp120 which is likely the target of HIV (Ezekowitz et al. 1989). MBL can also bind to Aspergillus fumigatus, Candida albicans (Neth et al. 2000) and protozoans such as Cryptosporidium parvum (Kelly et al. 2000); Plasmodium falciparum (Klabunde et al. 2002) and Trypanosoma cruzi (Kahn et al. 1996), to prevent infection.

**PHENOTYPES**

**MBL2 gene** (which encodes human MBL protein), along with other collectins, is a part of tightly linked cluster of genes found on the chromosome 10 (Guo et al. 1998). MBL deficiency is inherited in an autosomal co-dominant manner (Petittrew et al. 2009), with heterozygotes having about 10% of the normal functional level of MBL and homozygotes having less than 1% functional levels of MBL (Hibberd et al. 1999) and affect the serum concentration (Madsen et al. 1995, Madsen et al. 1998). The point mutations (single nucleotide polymorphisms, SNPs), three in exon 1 and two in promoter region of the MBL gene, lead to a dramatic decrease in the serum concentration of MBL. The transcription of MBL2 is regulated by two alternative promoters (named 0 and 1) where promoter 0 derived transcripts include an additional 5′ untranslated region (5′UTR) encoded by an extra exon (exon 0). Low extra-hepatic levels of MBL2 mRNA were predominantly found in small intestine and testis tissue, and were quantitatively dominated by promoter 1 transcripts. Moreover, these transcripts varied due to the use of alternative acceptor splice sites positioned inside exon 1 (Seyfarth et al. 2006).

Polymorphisms in Exon 1: The exon 1 mutations on the protein product are believed to impair oligomerization and lead to a functional deficiency. These point mutations are (now commonly referred to as B, C and D alleles) collectively denoted by ‘O’, with variant A indicating the wild type.

Variant B: A reported exon 1 mutation is at codon 54 in which glycine is replaced by aspartic acid (GGC to GAC) when studying a Eurasian population with frequency of approximately 25% (Sumiya et al. 1991). This mutation was associated with (most) low MBL serum levels, opsonization defect and recurrent bacterial infections.

Variant C: This exon 1 mutation is at codon 57 in which glycine is replaced by glutamic acid (GGA to GAA), in a sub-Saharan African population with frequencies of 50%–60% (Lipscombe et al. 1992).

Variant D: This exon 1 mutation is at codon 52 in which arginine is replaced by cysteine (CGT to CGG) (Madsen et al. 1994). This extra cysteine has been proposed to cause formation of adventitious disulphide bonds that hinder higher oligomer formation (Wallis and Drickamer 1999).

Polymorphisms in promoter region: Since exon 1 polymorphisms did not explain variations of MBL serum levels sufficiently, inter-individual variation in serum MBL levels revealed two polymorphisms (H/L and X/Y, at positions −550 and −221 respectively to transcription start site, TSS) in the upstream promoter region of the MBL2 (Madsen et al. 1995). The different combinations of these promoter polymorphisms result in different haplotypes, HY, LY, and LX, with high, medium, and low levels of MBL serum concentrations, respectively. Later a polymorphism, P/Q variant, at 5′UTR of the gene (part of Exon 1) was also identified (Madsen et al. 1998) which is also associated with low levels of serum MBL. Promoter polymorphisms and the exon 1 mutations cluster in a pattern of linkage disequilibrium (Garrard 2008, Verdu et al.)
The impact of the variations in MBL genotypes or serum concentrations on different human diseases has been intensively studied (disease association studies) (Sumiya et al. 1991, Kilpatrick 2002, Eisen and Minchinton 2003, Turner 2003). MBL deficiency is associated with an increased susceptibility to infection with *Neisseria meningitidis* (Bathum et al. 2006), and severity of atherosclerotic disease (Madsen et al. 1998). The recent findings have shown a correlation between MBL deficiency and *Pseudomonas* infections in cystic fibrosis patients, suggesting that MBL is inherently involved in clearance of potential pathogens in the body. MBL binding may facilitate the uptake of *Mycobacterium* by macrophages, thereby promoting infection. In contrast, presence of mutant alleles, which may lead to MBL deficiency, may convey a protective role against tuberculosis (TB) (Cosar et al. 2008, Thye et al. 2011, Singla et al. 2012). However, certain polymorphisms in *MBL2* contribute to development of TB in HIV patients (Raghavan et al. 2012, Alagarasu et al. 2007). MBL polymorphisms may also lead to systemic lupus erythematosus (Davies et al. 1995), Alzheimer's disease (Sjölander et al. 2013) and pulmonary disease in cystic fibrosis (Gabodle et al. 1999). MBL function may play a role in survival of kidney graft patients (Bay et al. 2013, Damman and Seelen 2013).

**MAJOR SITES OF EXPRESSION**

MBL is synthesized in the liver and circulates in the serum (Wild et al. 1983). However, extra-hepatic expression of MBL also observed (Nonaka et al. 2007). The expression of functional MBL peptide is largely genetically determined (see ‘Phenotypes’ section). MBL is considered as an acute phase reactant protein (serum levels increases during inflammation) (Ezekowitz et al. 1988) (see ‘Regulation of Concentration’ section). However, unlike other lectin proteins which increase drastically, MBL increases only 2-3 fold.

**SPLICE VARIANTS**

*MBL2* gene (which encodes MBL protein) is located on chromosome 10 (q11.2-q21) (Guo et al. 1998) with four exons and three introns. The gene encodes two major transcripts by alternative transcription, resulting in different lengths of mRNA transcripts. Transcription may initiate either at exon 1 or at an additional, non-coding 1kb upstream located, exon 0 (Naito et al. 1999, Sastry et al. 1989, Taylor et al. 1989). It is assumed that 10–15% of MBL in serum derives from exon ‘0’ transcription (Heitzeneder et al. 2012). Exon 1 encodes the signal peptide, a cysteine-rich region and part of the glycine-rich collagenous region, exon 2 encodes the remainder of the collagenous region, exon 3 encodes an α-helical coiled-coil structure, which is known as the ‘neck’ region, and exon 4 encodes the CRD, which adopts a globular configuration (Wallis et al. 2004, Madsen et al. 1995, Weis et al. 1992, Wallis 2007). The promoter region of the *MBL* gene contains a number of regulatory elements, which affect transcription of the protein (Dommett et al. 2006). Both, exon ‘0’ and exon 1, promoter regions possess a TATA box for transcription initiation. In both, the binding sites for transcription factors include response elements to IL-6. This finding was assumed to underlie the regulation of MBL synthesis as an acute phase protein. In addition, the promoter region of exon 1 comprises a glucocorticoid responsive element (Gabolde et al. 1999). However, human *MBL1* gene is a pseudogene.
<table>
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SUPPLEMENTARY

Supplementary information is available online.

REFERENCES


MOLECULE PAGE


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Wallis R (2007). Interactions between mannan-binding lectin and MASP5 during complement activation by the lectin pathway. Immunobiology, 212, 4-5.


This molecule exists in 33 states, has 32 transitions between these states and has 2 enzyme functions. (Please zoom in the pdf file to view details.)
Leukocyte surface antigen CD47

David R Soto Pantoja1, Sukhbir Kaur2, Thomas W Miller3, Jeffrey S Isenberg4, David D Roberts5

CD47, also known as integrin-associated protein (IAP), ovarian cancer antigen OA3, Rh-related antigen and MER6, is a widely expressed transmembrane receptor belonging to the immunoglobulin superfamily. CD47 is the counter-receptor for two members of the signal- regulatory protein (SHPS/SIRP) family and a high-affinity receptor for the secreted protein thrombospondin-1. Interactions with SIRP receptors play roles in self recognition and regulation of innate immune responses. Over-expression of CD47 on some cancers is a negative prognostic factor and protects against innate immune surveillance. Engagement of CD47 on vascular cells by thrombospondin-1 regulates calcium, cAMP, and nitric oxide/GMP signaling pathways that control blood pressure, tissue perfusion, and angiogenesis. Moreover, CD47 signaling in various cell types regulates pathways that can trigger cell death, limit stem cell self-renewal, regulate mitochondrial homeostasis and other differentiation pathways, and activate protective autophagy responses under tissue stress. On red blood cells CD47 is part of the Rh complex, but on other cell types it associates laterally in the membrane with integrins and specific signaling receptors. Impaired responses to cardiovascular stress and some pathogens in mice lacking CD47 and their enhanced survival of fixed ischemia, ischemia/reperfusion and radiation injuries identify important pathophysiological roles for CD47 in inflammatory responses and adaptation to stress.

KEYWORDS
Antigen identified by monoclonal antibody 1D8; Antigenic surface determinant protein OA3; CD47; CD47 antigen (Rh-related antigen, integrin-associated signal transducer); CD47 glycoprotein; CD47 molecule; IAP; Integrin associated protein; Integrin-associated protein; Integrin-associated signal transducer; Leukocyte surface antigen CD47; MER6; OA3; Protein MER6; Rh-related antigen

IDENTIFIERS

PROTEIN FUNCTION
Structure and history

CD47 was initially identified as an antigen that is missing in Rhesus (Rh)-null hemolytic anemia (Miller et al. 1987). Subsequent studies demonstrated that CD47 is not the primary cause of this disease but instead serves as a component of the Rh complex on red blood cells (RBC). The same protein was independently identified as the ovarian carcinoma tumor antigen OA3 and as a protein that co-purified with certain integrins and, therefore, named integrin-associated protein (IAP) (Campbell et al. 1992; Lindberg et al. 1993). In 1994 IAP and OA3 were shown to be identical to CD47 (Lindberg et al. 1994; Mawby et al. 1994). CD47 is a type I integral membrane protein composed of an extracellular immunoglobulin variable (IgV)-like domain, five membrane-spanning segments, and an alternatively spliced carboxyterminal cytoplasmic tail (Brown and Frazier 2001). The IgV-like extracellular domain is variably glycosylated with N-glycans and glycosaminoglycans and has a blocked amino terminus (Mawby et al. 1994; Kaur et al. 2011).

The IgV-like domain is linked via a long-range disulfide bond to a Cys residue on the extracellular loop between the 4th and 5th transmembrane segments. This disulfide is required for some signaling functions of CD47 (Rebres et al. 2001). A crystal structure for a recombinant form of the extracellular domain of CD47 has been published (Hatherley et al. 2008). However, the domain crystallized as a misfolded dimer with strand interchange between the two IgV domains, so its physiological relevance is unclear. A more relevant structure for this domain of CD47 was solved bound to a recombinant extracellular domain of its counter-receptor signal regulatory protein-α (SIRPα, also known as tyrosine phosphatase non-receptor type subtype-1, SHPS-1). Because some ligand-binding properties of CD47 have not been reproduced using the recombinant IgV domain and because of the absence of the long-range disulfide bond in the recombinant protein used for structure elucidation, the exact structure and orientation of CD47 on the cell surface remains uncertain.

Phylogeny

CD47 orthologs have been identified in all mammalian genomes sequenced to date, in birds including chicken, turkey, and zebra finch, and in the reptile Crotalus adamanteus (Eastern diamondback rattlesnake). However, no CD47 orthologs have been identified in fish or invertebrates, suggesting that CD47 originated in early land-dwelling vertebrates. Notably, eNOS(NOS3), which is a major vascular signaling target of CD47, also appeared at the same point in evolution (Toda and Ayajiki 2006). The extracellular domain of CD47 was reported to be distantly related to Drosophila melanogaster wrapper, an Ig-domain-containing GPI-anchored protein involved in neuron-glial interactions (Stork et al. 2009). The transmembrane domain of CD47 is related to the corresponding membrane domain of presenilin-1 (Watanabe et al. 2010).

Many members of the poxvirus family that infect vertebrate hosts (Chordopoxvirinae) including cowpox, vaccinia, variola (smallpox), Ectromelia virus, Myxoma virus, and lumpy skin disease virus encode CD47-like proteins, which are predicted to have been acquired from ancestral mammalian hosts some time before the divergence between rodents and primates (Hughes 2002). Some of these viral CD47 homologs are known to retain the capacity to bind SIRPα, which may explain the selective
pressure to maintain these in the poxvirus genomes. The Myxoma virus homolog M128L suppresses macrophage activation, apparently acting as a CD47 mimic when engaging SIRPα (Cameron et al. 2005).

Overview of signaling

CD47 serves as a counter-receptor for SIRPα, and signaling between these two receptors is bidirectional. Binding of CD47 initiates a signaling cascade in SIRPα-expressing cells that limits the phagocytic activity of macrophages, inhibits trafficking of dendritic cells, and regulates insulin-like growth factor 1 (IGF1) receptor signaling in vascular smooth muscle cells (Raymond et al. 2010; Maile and Clemmons 2003). Extending through the cytoplasmic domain of SIRPα has been extensively studied, and several recent reviews should be consulted (Oshima et al. 2002; van Beek et al. 2005; Barclay and Brown 2006; Matozaki et al. 2009). CD47 is also a counter-receptor for a second member of the signal-regulatory protein family, SIRPγ; this counter-receptor lacks a cytoplasmic domain, so the potential for signaling through it is unclear. Less attention has been given to understanding how SIRPα or SIRPγ binding alters signaling through CD47, which has been termed reverse signaling (Sarafati et al. 2008), and this remains a fertile topic for future research. Most studies of CD47 signaling to date have used its secreted ligand thrombospondin-1 (TSP1), TSP1-derived peptides that bind to CD47, or anti-CD47 antibodies to stimulate responses. There is also some evidence that secreted forms of SIRPα and SIRPγ can regulate the organization of neural synapses by engaging CD47 (Umemori and Sanes 2008), which suggests that SIRPα binding can also stimulate CD47 signaling responses.

An immobilized TSP1 peptide was used for the first affinity purification of CD47 (Gao et al. 1996a), but direct binding of native TSP1 to CD47 was only recently established (Isenberg et al. 2009). This peptide (4N1K, K106RFYVVMWK1024K) was derived from the C-terminal domain of TSP1 with added lysines to increase solubility and contains a Val–Val–Met motif that is required for its binding to CD47 (Gao et al. 1996a). Upon binding to CD47 4N1K is shown to inhibit human Thy T-cell differentiation by blocking production of IL-12 (Avice et al. 2001). In the same study this effect was corroborated using human antibodies against CD47, showing that this peptide regulates CD47-mediated development of Th1 cells with potential implications of CD47 in immune response. Furthermore, the 4N1K motif is repeated in a second peptide derived from the same domain that also binds to CD47 (7N3, 1102FIRVVMYEGKK1112). The role of these sequences in binding of native TSP1 to CD47 has been questioned because the VVM motifs are not surface exposed in the published crystal structure for the C-terminal domain of TSP1 (Kvansakul et al. 2004). However, flanking sequences in these peptides are surface exposed, and a computational modeling study predicted a potential conformational change in the C-terminal domain of TSP1 that could expose the VVM motif (Floquet et al. 2008). Experimental validation of this hypothesis is lacking, however, and these findings must be reconciled with evidence that glycosaminoglycan modification of CD47 at Ser64 (of mature protein) is necessary for TSP1 signaling (Kaur et al. 2011). TSP1 binding to CD47 may involve recognition of both protein and carbohydrate determinants.

Regulation of NO/cGMP signaling

Binding of TSP1 or the C-terminal signature domain of TSP1 to CD47 potently and redundantly inhibits nitric oxide (NO)/cGMP signaling in vascular cells (Isenberg et al. 2008a). This pathway is regulated via alterations in cytoplasmic calcium signaling and other undefined proximal signals that limit the activation of endothelial nitric oxide synthase (eNOS), soluble guanylate cyclase, and cGMP-dependent protein kinase (Isenberg et al. 2006a and 2006b; Isenberg et al. 2008a and 2008b; Bauer et al. 2010; Ramanathan et al. 2011). Inhibition of NO/cGMP signaling has been replicated using a recombinant C-terminal “signature” domain of TSP1, CD47-binding TSP1 peptides, and some CD47 antibodies (Isenberg et al. 2006a and 2006b; Isenberg et al. 2008a and 2008b; Ramanathan et al. 2011). This signaling pathway plays important roles in TSP1-mediated inhibition of angiogenesis, local vasoconstriction and systemic regulation of blood pressure, and promotion of platelet aggregation.

Heterotrimeric G protein signaling

Heterotrimeric G proteins are a second signaling target of CD47. Gi associates with CD47 in a detergent-resistant complex, and CD47 ligation by 4N1K results in increased GTP loading of Gi and decreased cytoplasmic cAMP levels (Frazier et al. 1999). This pathway regulates protein kinase A signaling in melanoma cells and platelets (Frazier et al. 1999), vascular smooth muscle cells, (Yao et al. 2011), T cells (Manna and Frazier 2003), breast carcinoma cells (Manna et al. 2005) and primary thyroid cells (Rath et al. 2006), but not in RBC (Brittain et al. 2004). In T cells, CD47 ligation by the TSP1 peptide 7N3 induces phosphorylation of the mitogen-activated protein (MAP) kinase Erk in a pertussis-toxin-sensitive manner, implying that Erk is one downstream target of CD47-induced heterotrimeric G protein signaling (Wilson et al. 1999). The downstream responses linked to this CD47 signaling pathway include altered cell adhesion, aggregation and survival.

Regulation of integrins

CD47 physically associates with and activates several integrins, including αβ3, αvβ3, αbβ1, and αβ1 (Gao et al. 1996b; Chung et al. 1997; Chung et al. 1999; Wang et al. 1999; Barazi et al. 2002). Activated integrins in turn can modulate a broad range of signaling pathways in cells (reviewed in Miranti and Brugge 2002; Luo et al. 2007; Askari et al. 2009). TSP1 peptides have most frequently been used as ligands to induce integrin activation via CD47. Some CD47 antibodies also modulate integrin activation. This response can be integrin-specific. For example, the CD47 antibody B6H12 inhibits peptide-mediated activation of αbβ1 integrin but directly activates αbβ1 integrin (Barazi et al. 2002). Activation of integrins may be important in some signaling functions of CD47. For example, knockdown of CD47 using a small interfering RNA (siRNA) inhibits collagen-induced cyclooxygenase-2 (Cox2) expression in intestinal epithelial cells through inhibiting association of CD47 with the collagen-binding integrin αbβ1 (Broom et al. 2009).

Cell death

The modified TSP1 peptide 4N1K induces death of various cell types, including human brain microvascular endothelial cells (Xing et al. 2009b), mouse cortical neurons (Xing et al. 2009a), four breast cancer cell lines (Manna and Frazier 2004), human monocytes and monocyte-derived dendritic cells (Johansson et al. 2004) and T cells (Manna and Frazier 2003). While the precise molecular mechanism of death induction by CD47 is
still unclear, the cell death induced by CD47 usually does not involve release of cytochrome c from mitochondria or activation of caspases. However it is known that in leukemic cells ligation of CD47 causes the induction of type III cell death associated signaling, including the induction of Drp1 translocation to the mitochondria (Bras et al. 2007). Also in leukocytes, cell death is preceded by a rapid depolarization of mitochondrial membrane potential (ΨΔm) (Manna and Frazier 2003; Manna and Frazier 2004; Saumet et al. 2005; Barbier et al. 2009; Merle-Beral et al. 2009).

Autophagy

CD47 is a potent regulator of autophagy in endothelial cells and T cells and in vivo in mice (Soto-Pantoja et al. 2012). The absence or suppression of CD47 expression increases some basal elements of the autophagy signaling pathway, and autophagy responses induced by stress such as ionizing radiation are markedly enhanced when CD47 is suppressed. CD47 regulates mRNA and protein expression of the upstream component of autophagy signaling beclin-1. This in turn regulates expression of several members of the ATG family, SQSTM1/p62, and the proteolysis and lipidation of LC3 (MAP1LC3A), a light chain of the microtubule-associated protein 1. Processed LC3 localizes to the autophagosome membrane and is essential for phagophore expansion. Induction of autophagy is necessary for the pro-survival effects of CD47 blockade in irradiated cells because siRNA suppression of ATG5 or ATG7 expression abrogated the survival advantage of irradiated CD47-deficient T cells. This pathway may also provide a mechanism for the ability of CD47 ligation to induce mitochondria-dependent cell death because beclin-1 interacts with and regulates Bcl-2.

Regulation of growth factor signaling

Based on co-immunoprecipitation and fluorescence resonance energy transfer studies, VEGFR2 is a proximal lateral binding partner of CD47 (Kaur et al. 2010). CD47 constitutively associates with VEGFR2 in the plasma membrane of endothelial cells, but ligation of CD47 by TSP1 and VEGFR2 by VEGF dissociates this complex. This dissociation inhibits VEGFR2 autophosphorylation and downstream signaling.

CD47 ligation also regulates activation of IGF1 receptor signaling in endothelial and vascular smooth muscle cells. Ligation of CD47 results in dissociation of CD47 from SIRPα (Maile and Clemmons 2003; Maile et al. 2003; Maile et al. 2012). This results in more sustained IGF1 receptor signaling due to a delay in the recruitment of the phosphatase SHP2. Furthermore, glucose-regulated cleavage of CD47 results in loss of SIRPα phosphorylation and Shc1 recruitment, which also decreases IGF1 receptor signaling (Maile et al. 2008). Hyperglycemia induces TSP1 expression, and this increased TSP1 protects CD47 from cleavage, which preserves CD47-SIRPα binding and results in increased IGF1 receptor signaling and increased phosphorylation of the β3 subunit of αvβ3 integrin (Maile et al. 2010)

Mitochondrial homeostasis

Young mice lacking either CD47 or TSP1 exhibit increased mitochondrial numbers and size in skeletal muscle (Frazier et al. 2011). This corresponded with increased levels of the mitochondrial proteins cytochrome c and VDAC1 and with increased mRNA levels encoding several mitochondrial proteins cytochrome c and VDAC1 and with increased levels of the mitochondrial membrane potential (ΨΔm) (Manna and Frazier 2003; Manna and Frazier 2004; Saumet et al. 2005; Barbier et al. 2009; Merle-Beral et al. 2009).

MOLECULE PAGE

The reported activities of 4N1K must be interpreted with caution except in cases where the activity has been replicated using native TSP1 and validated using anti-CD47 antibodies or CD47-deficient cells. Caution should be exercised in interpreting the peptide data alone because 4N1K was found to simulate aggregation and tyrosine phosphorylation in platelets from CD47-null mice (Tulase et al. 2001), and 4N1 and the related TSP1 peptide 7N3 increased integrin-mediated adhesion of CD47-deficient T cells (Barazi et al. 2002). Both of these activities were absent in control peptides in which the VVM motif was mutated to Gly–Gly–Met (GGM), indicating that this widely used control peptide is not sufficient to prove that a biological activity of these TSP1 peptides is mediated by CD47. Attention should also be paid to the concentrations of 4N1K used in published studies. The peptide has limited solubility in physiological buffers, and molecular aggregates may form at higher concentrations that generate nonspecific activities. In most of our studies, verifiable CD47-dependent activities of these peptides are observed at 1-5 μM concentrations. However, a majority of publications continue to employ concentrations exceeding 50 μM, which likely represent nonspecific activities even when the corresponding GGM control peptides are inactive at the same concentration.

REGULATION OF ACTIVITY

Ligation of CD47 by its physiological ligands TSP1, SIRPα and SIRPγ are the primary known mechanisms for regulating CD47 signaling. Most signaling studies published to date have used TSP1 or TSP1 peptides to modulate CD47 signaling. Because few studies have compared signaling induced by SIRPs and TSP1, it is currently unclear whether the pathways regulated by these ligands differ. Although TSP1 and SIRPα compete for
binding to CD47 on cells (Isenberg et al. 2009), we cannot conclude that they share a binding site or induce similar conformation changes or downstream signaling responses. Lateral interactions of CD47 with integrins, VEGFR2, and SIRPα also modulate signaling activities of CD47, and agents that perturb these interactions may be viewed as modulators of CD47 activity.

Post-translational modification of CD47 can positively and negatively regulate its signaling activity. Proteolytic cleavage of CD47 was reported to prevent its lateral association with SIRPα (Maile et al. 2008; Allen et al. 2009). Although direct evidence is lacking, binding to TSP1 should also be lost. Another unanswered question is whether the shed extracellular domain can still bind to SIRPα and/or CD47, and if so function as a dominant negative inhibitor of CD47 signaling or a diffusible agonist of SIRPα signaling on nearby cells. Another unanswered question is whether the transmembrane domain and cytoplasmic tail remaining after cleavage of the IgV domain have any signaling function.

Glycosylation of CD47 is another post-translational modification that regulates CD47 activity. Asparagine-linked glycosylation is necessary for surface localization of CD47 when heterologously expressed (Parthasarathy et al. 2006), but direct effects of N-glycosylation on CD47 ligand binding and signaling remain to be defined. Two serine residues on the extracellular domain are modified with chondroitin sulfate and heparan sulfate glycosaminoglycan chains (Kaur et al. 2011). This modification is necessary for inhibitory signaling by TSP1 in T cells. The same modification occurs on CD47 expressed by endothelial and vascular smooth muscle cells, and the signaling functions of TSP1 engaging CD47 on these cells may also require this glycosaminoglycan modification.

INTERACTIONS
Extracellular Ligands

SIRPα, SIRPγ and TSP1 are the best-characterized extracellular ligands of CD47. Interactions between recombinant extracellular domains of SIRPα and CD47 have been assessed using biophysical crystallographic, and mutagenesis studies that have identified specific residues in both proteins involved in this interaction (Nakaishi et al. 2008; Hatherley et al. 2008; Lee et al. 2007). High-affinity binding of SIRPα to CD47 is somewhat species-specific, and several variant residues that confer species specificity have been identified (Subramanian et al. 2006; Subramanian et al. 2007; Matozaki et al. 2009).

Although VVM motifs that bind CD47 in the context of synthetic peptides are conserved in all five thrombospondins, the recombinant C-terminal domains of TSP2 and TSP4 do not replicate the activity of the same domain of TSP1 to compete with the SIRPα extracellular domain for binding to cell-surface CD47 (Isenberg et al. 2009). Therefore, high-affinity binding to CD47 is specific for TSP1. CD47 is subject to post-translational modification with heparan sulfate and chondroitin sulfate chains at Ser64 and Ser79 (of mature protein) (Kaur et al. 2011). Interaction between TSP1 and CD47 and inhibitory signaling through CD47 in T cells requires modification of CD47 with a heparan sulfate chain at Ser64.

On the basis of its inhibition by CD47-blocking antibodies, fibrillar amyloid-β is another extracellular ligand for CD47 (Bamberger et al. 2003). Interaction with amyloid was proposed to involve a complex containing CD47, CD36, and α6β1 integrin. Anti-CD47 antibodies blocked phagocytosis of amyloid (Koenigsknecht and Landreth 2004), but recent studies indicate that amyloid inhibition of cGMP signaling does not involve a direct interaction with CD47 (Miller et al. 2010). Knockout and gene suppression studies indicate that amyloid interacts directly with the scavenger receptor CD36, and this interaction indirectly modulates signaling that requires CD47. Type IV collagen has also been proposed to be an extracellular ligand (Shahan et al. 2000), but proof of its direct interaction with CD47 is lacking.

There is a single report of homotypic binding of CD47 with itself, which could constitute another extracellular ligand to mediate cell-cell interactions (Rebres et al. 2005). Signaling consequences of this interaction remain to be defined.

Lateral membrane interactions

The primary lateral binding partners of CD47 in cells other than erythrocytes are integrins. At least in the case of α6β1, this interaction and the subsequent activation of the integrin requires the extracellular IgV domain, not the transmembrane domain of CD47 (Lindberg et al. 1996). Therefore, integrins should be considered specialized extracellular ligands of CD47. The CD47–integrin complex may also associate with other signaling proteins. Indeed, CD47 and integrin α6β1 form a complex with the tyrosine kinases Src, FAK and Syk (Chung et al. 1997). Furthermore, CD47 and integrin α6β1 can interact with heterotrimERIC Gi proteins (Frazier et al. 1999), as well as the tyrosine kinases Src, Lyn and the the tyrosine phosphatase SHP-2 (McDonald et al. 2004).

In endothelial cells the major signaling receptor for vascular endothelial growth factor (VEGFR2) was identifed as a proximal binding partner for CD47 (Kaur et al. 2010). Fluorescence resonance energy transfer studies indicted a close lateral interaction between CD47 and VEGFR2 that is dissociated when CD47 engages TSP1 and VEGFR2 engages VEGF. Dissociation of this complex inhibits signaling through VEGFR2, whereas signaling through VEGFR2 is enhanced in CD47 null cells.

Studies in smooth muscle cells imply that cis interactions may occur between the extracellular domain of CD47 and the extracellular domain of SIRPα expressed in the same cell (Maile et al. 2003; Maile et al. 2008). However, it is difficult to exclude the possibility that trans binding of shed extracellular domains of CD47 and/or SIRPα could account for the observed signaling responses. In either case, these studies show that signaling pathways engaged by the large cytoplasmic domain of SIRPα can mediate some responses to CD47 ligation when both are expressed by the same cells.

In T cells, CD47 is weakly associated with the apoptotic signaling receptor Fas, but activation or ligation of Fas promotes stronger association with CD47 (Manna et al. 2005). Cytoplasmic binding partners

Heterotrimeric Gi link in CD47 signaling to the cytoskeleton of responsive cells (Gao et al. 1996b). GTP loading of Gi is responsive to CD47 ligation (Frazier et al. 1999). This was proposed to involve a molecular complex of CD47, containing 5 transmembrane segments, with an integrin (which has two transmembrane segments) to form a functional seven-
transmembrane-domain complex that could activate Gi (Frazier et al. 1999). Although an attractive hypothesis, this model has not been confirmed experimentally. The cytoplasmic tail of CD47 also interacts with protein linking IAP and cytoskeleton-1 (PLIC-1) and PLIC-2 (Wu et al. 1999). PLIC-1 was later shown to interact with Gβγ and so may mediate their interaction with CD47 alone or in the context of an associated integrin (N’Diaye et al. 2003).

Some signaling through CD47 seems to be independent of G proteins, and additional ligands for the cytoplasmic tail of CD47 may mediate such signals. BNIP3 was identified as a binding partner for the transmembrane domain of CD47 by a yeast two-hybrid screen (Lamy et al. 2003; Lamy et al. 2007). Ligation of CD47 can trigger translocation of BNIP3 to mitochondria where it can trigger programmed cell death. In pulmonary arterial endothelial cells CD47 may also interact with caveolin-1 to control endothelial nitric oxide synthase activity under hypoxic conditions (Bauer et al. 2012).

PHENOTYPES

Angiogenesis

Plasma levels of the soluble CD47 ligand thrombospondin-1 (TSP1), and putatively activated CD47, correlated strongly with the presence of peripheral arterial disease in patients (Smadja et al. 2011). In culture systems human endothelial cell outgrowth, as a marker of angiogenic activity, is inhibited by a CD47 activating peptide (Xing et al. 2009).

Cellular energetics

The cyclic nucleotide second messengers cGMP and cAMP drive an array of important pro-survival pathways in cells. In human Jurkat cells the C-termus domain of TSP1, that specifically binds CD47, inhibits soluble guanylyl cyclase (Ramanathan et al. 2011). Conversely, β-amyloid inhibition NO-mediated activation of sGC is CD47 dependent in Jurkat cells (Miller et al., 2010). The CD47 ligand TSP1 limits adenylylate cyclase driven production of cAMP in human arteriel vascular smooth muscle cells (VSMC) (Yao et al. 2011). Also the CD47 binding domain of TSP1, E123CaG1 inhibits NO-mediated adhesion of human VSMC (Isenberg et al. 2009a). In human umbilical vein endothelial cells the CD47 activating peptide (7N3) inhibited NO-mediated increases in cell adhesion whereas the CD47 binding domain of TSP1 inhibited NO-stimulated production of cGMP in VSMC (Isenberg et al. 2006). CD47 signaling also limits mitochondrial biogenesis in some tissues including in skeletal muscle, and consequently limits exercise endurance (Frazier et al. 2011). Interestingly the metabolic advantage of CD47 null mitochondria diminishes with age.

Hematology

In human platelets CD47 promoted aggregation through limiting NO anti-aggregatory effects (Miller et al. 2010; Isenberg et al. 2008) based on experiments with CD47 null cells. Also human platelet adhesion to activated endothelial cells was decreased by blocking CD47 with antibody B6H12 (Lagadec et al. 2003). Platelet harvesting techniques can alter expression levels in stored platelets (Albanyan et al. 2009), whereas microparticles that are shed from stored platelets express CD47 (Sadallah et al. 2011). In human immune modulating cells a CD47 blocking antibody inhibited the expression of the costimulatory molecules and MHCII molecules on dendritic cells (Yu and Lin 2005), and a CD47 antibody decreased human T cell proliferation in a mixed lymphocyte cell preparation (Seiffert et al. 2001). Osteoclast formation stimulated by human myeloma cells was limited by agents that suppress activation of CD47 (Kukreja et al. 2009). In ITP derived human cells, a CD47 antibody decreased macrophage scavenging of platelets (Catani et al. 2011). A CD47 antibody limits human red blood cells interaction with fibrinogen (De Oliveira et al. 2012).

In addition to being a component of the Rh antigen complex on red blood cells, functional roles for CD47 have been identified on erythrocytes. CD47 was identified as an adhesion receptor on sickle erythrocytes that mediates adhesion to immobilized TSP1 (Brittain et al. 2001). This was subsequently shown to involve activation of α4β1 integrin (Brittain et al. 2004). Ligation of CD47 on erythrocytes by peptides, TSP1 or a CD478 antibody was shown to induce expression of phosphatidylserine (Head et al. 2005). Recently, CD47 was identified as both an inhibitor and stimulator of erythrocyte phagocytosis (Burger et al. 2012).

Tissue healing

A role for CD47 is human disease is inferred from the ability to protect human cells from hypoxia with CD47 blocking antibodies (Bauer et al. 2012) and radiation injury with CD47 suppressing morpholinos (Maxhimer et al. 2009) and in preclinical animal models. Random ischemic mycocytean flaps and full-thickness skin grafts heal faster and demonstrate greater blood flow in CD47-null mice (Isenberg et al. 2007a; Isenberg et al. 2008b). Similarly, CD47-null mice tolerate hind limb ischemia significantly better even in the face of advanced age (Isenberg et al. 2007b). Targeting CD47 with antibodies or a translation-blocking antisense morpholino oligonucleotide can increase blood flow and tissue survival following ischemia (Isenberg et al. 2007c).

Bone and joint


Ischemia-reperfusion (I/R) injury

I/R injury is a common mechanism behind the pathophysiology of peripheral vascular disease, complicates recovery from traumatic injuries and limits the success of organ transplantation. Hypoxia, as a mimic of IR injury, upregulates CD47 in renal tubular epithelial cells (Rogers et al. 2012) and pulmonary arterial endothelial cells (Bauer et al. 2012). Lack of CD47 is associated with significant resistance to I/R injury in both a liver I/R injury model (Isenberg et al. 2008a) and in renal I/R injury (Rogers et al. 2012). Therapeutic inhibition of CD47 shows a benefit in a rat soft tissue I/R injury (Maxhimer et al. 2009) and in liver and renal I/R injury (Isenberg et al. 2008a; Rogers et al. 2012). Therefore, CD47 is an important mediator of I/R injury responses and a promising therapeutic target.

Cardiovascular control
Pulmonary arterial hypertension (PAH) is a progressive fatal disease marked by a loss of lung vascular response to vasodilators (including NO) and over-active vasoconstrictor responses. Analysis of lung parenchyma from PAH patients has found significant induction of CD47 and TSP1 (Bauer et al. 2012). Antibody blockade of activated CD47 mitigates PAH in a pre-clinical model whereas mutant mice lacking the high affinity CD47 ligand TSP1 are protected from hypoxia-mediated PAH (Bauer et al. 2012).

CD47 also regulates systemic vascular cell responses. In human arterial endothelial cells activated CD47 limits endogenous NO production by endothelial nitric oxide synthase (eNOS) and in this manner inhibits arterial vasodilation (Bauer et al. 2010). In animals intravenous TSP1, by activating CD47, acutely elevates blood pressure (Bauer et al. 2010).

CD47-null mice show enhanced NO sensitivity and cGMP signaling. This also translates into dramatic increase in regional blood flow following an NO challenge and compensatory alterations in blood pressure (Bauer et al. 2007a; Isenberg et al. 2009b). Likewise, cardiac function responses to vasodilators are enhanced in the CD47-null mice (Isenberg et al. 2009b), whereas CD47 are hypotensive at rest (Bauer et al. 2010).

Host defense

In a subset of patients with hepatitis C infection liver biopsies showed upregulation of CD47 (Hevezi et al. 2011). Interestingly monocyte CD47 expression decreased following burn injury and correlated positively with multi-organ failure (Wang et al. 2011). Both CD47 and TSP1 are upregulated in skeletal muscle biopsies from patients with inclusion body myositis (Salajegheh et al. 2007). CD47-null mice display increased susceptibility to Escherichia coli peritonitis compared with heterozygote controls (Lindberg et al. 1996).

CD47-null macrophages show less ability to phagocytose Coxiella burnetii (Capo et al. 1999). With advanced age CD47-null mice occasionally develop cheek abscesses (our unpublished results). Conversely, CD47-null mice are protected from LPS-triggered acute lung injury and E. coli pneumonia (Su et al. 2008). A sterile inflammation induced by oxazolone takes longer to resolve in CD47-null mice (Lamy et al. 2007). Neutrophils derived from CD47-null murine bone marrow had only a quarter of the sensitivity toward an agonist of the Toll-like receptor dimer TLR2-TLR6 (Chin et al. 2009).

Nervous system

CD47 is expressed in myelin, mast cells and astrocytes from human multiple sclerosis lesions (Han et al. 2012) and CD47 null mice are protected from experimental autoimmune encephalomyelitis. A TSP1-derived peptide, 4N1K, that reportedly activates CD47, induced cell death in human brain microvascular endothelial cells (Xing et al. 2009). Conversely, the 4N1K peptide is reported to upregulate VEGF and MMP-9 in human brain microvascular endothelial cells and astrocytes (Xing et al. 2010). Also several miRNAs have been found to be increased in multiple sclerosis lesions and associated with decreased astrocyte CD47 (Junker et al. 2009). In human monocytes and rodent microglia β-amyloid stimulated signaling occurred through CD47 and several other cell surface proteins (Bamberger et al. 2003). These findings are made more relevant by a report that β-amyloid, via CD47, can limit vascular cell NO signaling (Miller et al. 2010). Dendritic outgrowth is increased in CD47 overexpressing cells (Numakawa et al. 2004), and CD47-null mice have decreased memory retention compared with wild-type controls (Chang et al. 1999). Similarly, injection of the anti-CD47 antibody miap301 into the dentate gyrus of the hippocampus in mice impaired their memory retention (Chang et al. 2001). In addition, cultured neurite development was decreased in cells from CD47-null mice (Murata et al. 2006).

Radiation injury

Human vascular endothelial cells treated with a CD47 targeting morpholino oligonucleotide are protected from radiation injury and death (Maxhimer et al. 2009). Skin, muscle and bone marrow in CD47-null mice are highly resistant to radiation injury (Isenberg et al. 2008a). Vascular cells isolated from the null mice or from mice lacking the CD47 ligand TSP1 show similar resistance to radiation-induced killing, indicating that this process is cell autonomous. Consistent with the null phenotype, antisense suppression of CD47 produces similar radioprotection of skin, muscle and bone marrow hematopoietic progenitor cells in wild-type mice (Maxhimer et al. 2009).

Immune responses

CD47 plays multiple roles in innate and adaptive immunity. There is a deficit in the ability of CD47 null mice to mount an immune response to particulate antigens such as RBC, and the response to soluble antigens is normal (Hagnerud et al. 2006). CD47-null mice show increased sensitivity to intraperitoneal E. coli that is secondary both to delayed polymorphonuclear leukocyte migration to the site of infection and to defective activation at the site (Lindberg et al. 1996). However, CD47-null mice are resistant to a similar challenge of bacteria to the lungs (Su et al. 2008). The increased susceptibility of CD47-null mice to intraperitoneal E. coli infection as well as other bacterial and viral infections is associated with defective transendothelial migration of neutrophils (Lindberg et al. 1996; Herold et al. 2006). Transendothelial migration of neutrophils, monocytes, and T cells is limited in CD47 null mice, and the failure of wild type bone marrow transfer to correct this defect suggested that CD47 on endothelium is important for leukocyte egress (Azcutia et al. 2012). Binding of the human CD47 function blocking antibody B6H12 to endothelial cells induces cytoskeletal remodeling and up-regulates Src and Pyk2 tyrosine kinase, increasing tyrosine phosphorylation of VE-cadherin, an inducer of cell adhesion. Thus, endothelial cell CD47 is important for leukocyte recruitment during the course of infection.

The interaction between SIRP-α on phagocytes and CD47 on target cells inhibits phagocytosis and constitutes a species-specific self-recognition mechanism (Matozaki et al. 2009). Wild type mice rapidly eliminate CD47-deficient RBC, transfused porcine RBC, T cells, and bone marrow independent of antibody or complement stimulation (Oldenburg et al. 2000; Blazar et al. 2001; Wang et al. 2011). Expression of human CD47 on porcine RBC suppresses their phagocytosis by human macrophages (Ide et al. 2007). Thus, tumor cells with elevated CD47 expression are more resistant to being cleared by phagocytosis.

CD47 regulates T cell activation. CD47 ligation by certain antibodies or by TSP1 peptides has effects similar to those induced by CD28 ligation on T cell activation and proliferation.
Widespread expression of CD47 is believed to be important for T cells, and the inhibitory effects of re-expressing CD47 by TSP1 decreased c-Myc expression in WT human CD47-null mice and in human T cells lacking CD47. Ligation of CD47 by TSP1 binding to CD47 inhibits the ability of T cells to respond to exogenous hydrogen sulfide and inhibits the induction of endogenous hydrogen sulfide biosynthesis in response to T cell receptor signaling (Miller et al. 2013).

CD47 is a negative regulator of T cell activation along the Th1 lineage (Avice et al. 2000). CD47 antibody B6H12 inhibited the development of Th1 differentiated cells and down-regulated production of IFN-γ (Avice et al. 2000). The CD47 antibody B6H12 inhibited the development of Th1 cells and down-regulated production of IFN-γ (Avice et al. 2000). CD47-null mice on a Th2-prone BALB/c background develop Th1-biased cellular and humoral responses (Bougoumouh et al. 2008). This bias is consistent with the exaggerated contact hypersensitivity response in CD47 deficient mice (Bougoumouh et al. 2008; Lamy et al. 2007). In addition to effects on T cell polarization, however, TSP1 null mice show a similar hypersensitivity response, which was attributed to a deficiency in CD47-mediated T cell apoptosis (Lamy et al. 2007).

Stem cell self-renewal

Differentiated cells can be reprogrammed into pluripotent stem cells by elevating expression of the transcription factors c-Myc, Sox2, Oct4, and Klf4. However, primary endothelial cells cultured from the lungs of CD47-null mice spontaneously underwent reprogramming to multipotent stem cells when deprived of serum (Kaur et al. 2013). The same cells from WT mice became senescent and stop proliferating. This property of CD47-null mice on a Th2-prone BALB/c background develop Th1-biased cellular and humoral responses (Bougoumouh et al. 2008). This bias is consistent with the exaggerated contact hypersensitivity response in CD47 deficient mice (Bougoumouh et al. 2008; Lamy et al. 2007). In addition to effects on T cell polarization, however, TSP1 null mice show a similar hypersensitivity response, which was attributed to a deficiency in CD47-mediated T cell apoptosis (Lamy et al. 2007).

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Promoter and transcriptional regulation

A core promoter for the CD47 gene was localized between nucleotide positions –232 and –12 relative to the transcription initiation codon in two tumor cell lines, a range that contains several canonical sites for transcription factor binding, including sites for nuclear respiratory factor 1 (NRF-1; also called Pal or α-Pal) and sites for SP1 and EGR1 (Chang and Huang 2004). Using reporter constructs, evidence was presented for a functional role of the NRF-1 site. Antisense suppression of CD47 was subsequently shown to inhibit neurite outgrowth in primary mouse cortical neurons induced via NRF-1, implying that NRF-1 positively regulates CD47 expression in cortical neurons (Chang et al. 2005).

Thus, CD47 plays a major role in limiting the activation of innate inflammatory pathways. Red blood cells deficient of CD47 are removed by circulating splenic macrophages (Oldenborg et al. 2000). On the other hand expression of CD47 on normal red blood cells inhibits this removal. The prevention of elimination is regulated by interactions with inhibitory receptor signal regulatory protein alpha (SIRPα). In humans these interactions may account for high CD47 expression being a negative prognostic factor for many solid tumors.

**SPLICE VARIANTS**

Human and mouse CD47 transcripts are both subject to alternative splicing such that additional short exons are added to the 3’ end of the mRNA (Reinhold et al. 1995). Four splice variants are known, the shortest form (form 1) ending in the amino acid sequence MKFVE. Each variant is successively longer by 12 (form 2), 7 (form 3) and 12 (form 4) amino acids, and in each case, the amino acid at the C terminus of the shorter form is altered by the new nucleotide sequence at the splice junction when the next longest form is created.

**REGULATION OF CONCENTRATION**

An additional splice isoform lacking 21 amino acids in the extracellular domain near the first transmembrane region was first identified in a cDNA library from the MSS62 mouse spleen stromal cell line (Furusawa et al. 1998). This isoform was found to be widely expressed. The same isoform was obtained by expression cloning from an adult mouse brain cDNA library using SIRPα as a bait, indicating that this isoform retains SIRP binding (Jiang et al. 1999).

Micro-RNAs regulate target mRNA causing degradation and/or inhibition of translation and are emerging as important regulators of CD47 expression. It has been reported that miR-133 regulates CD47 in vitro in Esophageal Squamous Cell Carcinoma (ESCC). Moreover RT-PCR analysis of human tumors indicates low expression of miR-133 in lesions where CD47 was elevated (Suzuki et al. 2012). Furthermore, Three
microRNA upregulated in active multiple sclerosis lesions (microRNA-34a, microRNA-155 and microRNA-326) targeted the 3'-untranslated region of CD47 in reporter assays, with microRNA-155 even at two distinct sites (Junkers et al. 2009).

CD47 Gene Polymorphisms

Cross-species polymorphisms in CD47 confer species-restricted recognition by SIRPα (Subramanian et al. 2006). Within humans, however, CD47 is remarkably free of coding polymorphisms. This implies a strong selective pressure to limit divergence of CD47 within species, which might be associated with its role in self-recognition by the innate immune system (van den Berg and van der Schoot 2008).

However, polymorphisms that alter the expression of CD47 have recently been reported to contribute to its dysregulation in cancer. An association study identified polymorphisms in CD47 and other extracellular matrix pathway genes as putative prognostic markers for colorectal cancer (Lascorz et al. 2012). A SNP in CD47 (rs12695175 CC versus AA) was associated with altered expression, patient survival of colorectal cancer (HR = 2.18, 95 % CI 1.10-4.33), and with overall survival (HR = 1.99, 95 % CI 1.04-3.81). Three additional SNPs in CD47 (rs9879947, rs3804639, and rs3206652 in the 3′-UTR) were associated with the occurrence of distant metastasis. Of these, rs3804639 also altered the expression of CD47.

Physiological and pathological regulation of CD47

Increased CD47 expression was noted in the brain hippocampus during postnatal development and in cultured hippocampal neurons maturing in vitro (Ohnishi et al. 2005).

Under normal static culture conditions, human umbilical vein endothelial cells (HUVECs) express high levels of CD47 on their surface. When such cultures are subjected to laminar flow in a parallel plate flow chamber, expression of CD47 decreases, but it is maintained in regions of turbulent flow (Freyberg et al. 2000; Freyberg et al. 2001). The expression of CD47 (along with TSP1) was found to increase the apoptotic index of the HUVEC monolayer. This TSP1–CD47 induction of apoptosis was also extended to fibroblasts (Graf et al. 2002).

CD47 expression is regulated in the adaptive immune response during T cell activation. Pre-clinical data indicates TSP-1 through CD47 can inhibit T-cell receptor signaling (Kaur et al. 2011). Human CD4 T-cells that show a change in conformation that impairs SIRP-α-Fc binding of CD47 can become sensitized to death by TSP-1 during the contraction phase of the T-cell activation response. However, release of IL-2 reverses the phenotype and increases CD47 expression which prevents clearance by macrophages and reduces sensitivity of death by TSP-1 binding (Van et al. 2012). This demonstrates that the regulation of expression of CD47 in humans is key in controlling the balance of inflammatory responses. Furthermore, accumulation of CD4 effector cells in the lymph nodes and at mucosal sites of Crohn’s disease patients demonstrate CD47 elevated expression independent of TSP-1 levels in colon tissue (Van et al. 2012). Spontaneous apoptosis of neutrophils was associated with decreased cell surface CD47 expression and more rapid ingestion by monocyte-derived macrophages than neutrophils with high CD47 expression (Lawrence et al. 2009).

In multiple sclerosis lesions mRNA and protein levels of CD47 are reduced (Han et al. 2012) indicating that the regulation of CD47 during pathogenesis may be tissue and disease dependent. Moreover, low expression of CD47 has been reported during immune thrombocytopenia, the reduction of platelets is not due to clearance by macrophages due to low CD47 and SIRPα interactions but due to increased apoptosis due to faulty CD47 signaling (Catani et al. 2011).

Hemophagocytic lymphohistiocytosis is an autosomal recessive disorder that is associated with parenteral consanguinity. Kuriyama et al. identified a role for CD47 down-regulation of CD47 in hematopoietic stem cells that stimulates phagocytic clearance of these stem cells in hemophagocytic lymphohistiocytosis (Kuriyama et al. 2012).

Preclinical studies indicate that CD47 results in the protection of soft tissues to ischemia reperfusion injury (IRI) (Soto-Pantoja et al. 2012). This is due in part to increased blood flow, reduction in inflammation and decreased generation of reactive oxygen species. Along this same line it is known that TSP1, via CD47, inhibits eNOS activation and endothelial-dependent arterial relaxation conversely increasing blood pressure burden (Bauer et al. 2010). In human studies individuals with pulmonary arterial hypertension (PAH) show high-level expression of TSP1 and CD47 in the lungs and elevated expression of TSP1 and activated CD47 in experimental models of PAH. Further assessment of human endothelial cells indicated that activation of CD47 is due to hypoxia.

On the other hand elevated expression of CD47 is inversely associated with the onset of multiple organ dysfunction syndrome (MODS). Flow cytometry analysis of circulating monocytes of burn patients indicated a reduction of CD47 expression and the severity of MODS in these subjects (Wang et al. 2011).

Regulation of CD47 turnover

Studies of the CD47 ligand TSP1 on T cells revealed a rapid increase in TSP1 surface expression following T-cell activation, and blocking either CD47 or TSP1 inhibited integrin-dependent T-cell adhesion (Li et al. 2006). TSP1 bound to CD47 on T cells was rapidly internalized; this involved another TSP1 receptor, CD91. This study did not examine whether CD47 internalizes with the bound TSP1, but this question merits further study. Loss of cell-surface CD47 was also implicated in the clearance of senescent RBC on the basis of a 30% decrease in CD47 expression in aged RBC (Khandelwal et al. 2007). Because transient down-regulation of CD47 expression has such profound effects on tissue responses to ischemic injuries, the potential for physiological down-regulation of CD47 deserves more attention.

CD47 is also regulated during hyperglycemia. Hyperglycemic conditions induced protection of CD47 from cleavage which maintains the ability of hyperglycemia to enhance IGF-1 signaling. This cleavage of CD47 is mediated by matrix metalloproteinase-2 and is inhibited by elevated expression of TSP-1 during hyperglycemia (Maile et al. 2010).

Regulation by cytokines

Changes in CD47 expression in response to cytokines was studied in four ovarian cancer cell lines (Imbert-Marcille et al. 1994). Interferons and TNFα individually increased CD47 expression, but more dramatic increases were seen when TNFα
or interferon-α were combined with interferon-γ.

Cancer

Malignant transformation in ovarian cancer was the first pathological context in which elevated CD47 expression was noted (Poels et al. 1986). Using the antibodies OV-TL3 and OV-TL16, CD47 expression was confirmed to be elevated in ovarian adenomas, carcinomas, endometroid, clear cell and mixed Müllerian carcinomas, leading to the conclusion that CD47 is a pan-ovarian-carcinoma antigen (Van Niekerk et al. 1993). Exposure of rats to an iron chelate as a renal carcinogen was found to increase CD47 expression in kidney, and elevated CD47 was also found in high-grade renal cell carcinomas and their lung metastases (Nishiyama et al. 1997). More recently, increased CD47 expression was noted in four of five rat prostate cancer cell lines (Vallbo and Damber 2005), human multiple myelomas (Rendtiew Danielsen et al. 2007), T-cell acute lymphoblastic leukemia versus T-cell lymphoblastic lymphoma (Raetz et al. 2006), oral squamous cell carcinoma (Suhr et al. 2007), human acute myeloid leukemia-associated leukemia stem cells (Majeti et al. 2009) and CD44+ tumor-initiating bladder carcinoma cells (Chan et al. 2009). Analysis of patients with malignant myeloma indicated that bone marrow cells overexpress CD47 when compared with non-myeloma cells in over 70% of patients (Kim et al. 2012). CD47 is over-expressed in additional solid tumors including those from kidney, lung, breast, colon, and stomach (Willingham et al. 2012). Those solid tumors with higher CD47 expression had poorer prognosis.

Increased CD47 expression correlates with an ability to evade phagocytosis by macrophages and cytolyis by NK cells (Jaiswal et al. 2010). Kim et al. found that elevated expression of CD47 on head and neck squamous carcinoma cells inhibited natural killer (NK) cell-mediated cytoxicity and that the CD47-neutralizing antibody B6H12 enhanced NK-mediated cytotoxicity (Kim et al. 2008). Similar therapeutic effects of the same CD47 antibody were subsequently reported to increase macrophage-mediated phagocytosis of acute myeloid leukemia (AML) stem cells and bladder carcinoma tumor initiating cells (Jaiswal et al. 2009; Chan et al. 2009), and therapeutic effects of the CD47 antibody B6H12 were shown against a human AML xenograft in immune-deficient mice lacking T cells, B cells and NK cells (Majeti et al. 2009). Thus, one function of the widely reported elevation of CD47 expression in cancer appears to be protection against host immune surveillance.

Pharmacological Regulation

Mevinolin is a cholesterol-lowering drug that was found to induce CD47 expression in multipotent bone marrow stromal (D1) cells (Kim et al. 2009). Specific suppression of CD47 expression in vitro and in vivo can be achieved using antisense morpholino oligonucleotides that target the 5’-region of its mRNA (Soto-Pantoja et al. 2012).

**ANTIBODIES**

Table 1. Commonly used monoclonal antibodies specific for human CD47

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Source</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/1A4</td>
<td>Mouse IgG1 reacts with human CD47</td>
<td>(flow cytometry)</td>
</tr>
</tbody>
</table>

**MOLECULE PAGE**

Mouse IgG2b Reacts with human CD47

Sources: GenWay

1F7 (induces T cell and breast cancer cell apoptosis)

Mouse IgG1 reacts with human CD47

Sources: Santa Cruz

2D3 (co-stimulates T cells, protects from apoptosis)

Mouse IgG1 reacts with human CD47

Sources: eBioscience, American Type Culture Collection

2E11 (co-stimulates T cells)

Mouse IgG1 reacts with human CD47

Source: American Type Culture Collection

Ad22 (induces T cell apoptosis)

Source: Rolf Petterson (Norway)

B6H12 (Human monoclonal antibody, function blocking for SIRPα and TSP1 binding, inhibits NO-stimulated cGMP signaling, blocks TSP1 inhibition of T cell receptor signaling, inhibits αβ3 integrin activation but increases α4β1 integrin activation, radioprotection of endothelial cells, induces apoptosis of B and T cells when immobilized, increases NK cell- and macrophage-mediated tumor cell killing; can be used in western blot, immunoprecipitation, flow cytometry)

Mouse IgG1 reacts with human protein

Sources: American Type Culture Collection, Abcam, eBioscience, Santa Cruz, Labvision, Novus Biologicals

**BRIC125**

Mouse IgG2b reacts with human CD47

Source: Anstee

BRIC126 (western blot, immunoprecipitation, flow cytometry)

Mouse IgG2b reacts with human, bovine, porcine CD47

Sources: Millipore, RDI, Santa Cruz, Hoelzel-biotech, LifeSpan Biosciences

CIKM1 (blocks monocyte trafficking, inhibits NO-stimulated cGMP signaling, co-stimulates AP1 activity and CD69 expression in T cells when immobilized)

Mouse IgG1 reacts with human CD47

Sources: BD Biosciences Pharmingen, Hoelzel-biotech

**CC2C6**

Mouse IgG1 reacts with human CD47

Sources: Santa Cruz, Biolegend

HCD47

Mouse IgG1 reacts with human CD47
MEM122
Mouse IgM reacts with human, primate, porcine CD47
Sources: Santa Cruz, Abcam, Exbio, Cedarlane Labs
OVTL3 (Fab’2 fragment used for imaging human ovarian tumors)
Mouse IgG1 reacts with human CD47
Source: V. Zurawski, Centocor
OVTL16
Mouse IgG1 reacts with human CD47
Source: Santa Cruz
<table>
<thead>
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<th>STATE DESCRIPTION</th>
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<tr>
<td>CD47</td>
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<tr>
<td>CD47-N16,55,93G3</td>
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<tr>
<td>CD47-pyroGlu (blocked)</td>
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<td>cell surface</td>
<td>Frazier WA et al. 1999</td>
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<tr>
<td>CD47/aVβ3/Gαi2-GTP</td>
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<td>Lamy L et al. 2003</td>
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<td>cell surface</td>
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</tr>
<tr>
<td>CD47/PLIC</td>
<td>cell surface</td>
<td>N'Diaye EN and Brown EJ 2003</td>
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<td>CD47-chondroitin sulfate</td>
<td>cell surface</td>
<td>Kaur S et al. 2011</td>
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ACKNOWLEDGEMENTS
This work was supported by the Intramural Research Program of the NIH/NCI (D.D.R.), 1RO1HL08954-01 (NIH/NHLBI), 1PO1HL103455-01 (NIH), 11BGIA7210001 (AHA), the Institute for Transfusion Medicine, and the Western Pennsylvania Hemophilia Center (J.S.I.).

SUPPLEMENTARY
Supplementary information is available online.

REFERENCES


J Biol Chem, 285,


Nakaishi A, Hirose M, Yoshimura M, Oneyama C, Saito K, Kuki N,


This molecule exists in 30 states, has 31 transitions between these states and has 0 enzyme functions. (Please zoom in the pdf file to view details.)
L-Ficolin

Anjana Chandrasekhar1, Ashok Reddy Dinasarapu1, Steffen Thiel2, Shankar Subramaniam1

L-ficolin is a serum lectin synthesized (as a ~37 kDa polypeptide) predominantly by the liver, and is one of the key molecules of the innate immune system. It has an amino (N)-terminal cysteine-rich region, a middle stretch of a collagen-like sequence, and a fibrinogen-like domain in the carboxy (C)-terminus. Three identical polypeptides form a structural (triple helical) subunit, with the help of the collagen-like domain. Further oligomerization of this subunit results in different sized L-ficolin molecules (from dimers to tetramers) in circulation. However, the tetrameric form (composed of 12 polypeptides) is the most prevalent structure. The polypeptides in the structural subunit are cross-linked by disulphide bonds in the N-terminal region. The fibrinogen-like domain forms a globular structure. The overall structure of oligomeric L-ficolin closely resembles mannose-binding lectin (MBL). Similar to MBL, L-ficolin also acts as a pattern recognition receptor. It primarily recognizes acetylated sugar residues on the cell surface of different gram-positive and gram-negative bacteria, viruses and other pathogens. There are two pathways by which L-ficolin may participate in a host defense response: 1) It activates the complement lectin pathway, via MBL/ficolin associated serine proteases (MASPs), that converges with the classical complement pathway at the level of complement C4, and 2) it may also act directly as an opsonin, enhancing phagocytosis by binding to cell-surface receptors present on phagocytic cells. M-ficolin and H-ficolin are structurally similar to L-ficolin. However, they differ in their tissue expression and binding affinities to pathogenic ligands.

KEYWORDS
37 kDa elastin-binding protein; Collagen/fibrinogen domain-containing protein 2; EBP-37; FCN2; FCNL; Ficolin (collagen/fibrinogen domain containing lectin) 2 (hucolin); Ficolin B; Ficolin-2; ficolin-2; Ficolin-B; Ficolin-beta; Hucolin; L-ficolin; L-Ficolin; P35; Serum lectin p35

PROTEIN FUNCTION
L-ficolin, originally discovered as a corticosteroid binding protein (Edgar 1995) is recognized as an important player in the lectin pathway of complement activation (Ali et al. 2012, Kilpatrick and Chalmers 2012). The original finding of the protein was due to the binding of L-ficolin to cyogen bromide (CNBr)-activated Sepharose and was thus not due to specific binding to the proteins coupled to the beads. Its oligomeric structure closely resembles that of other two ficolins (M-ficolin and H-ficolin), collectins such as MBL and also that of the recognition molecule of the classical pathway, C1q (Garred et al. 2010). L-ficolin exhibits a ~37 kDa polypeptide chain with an N-terminal cysteine rich region, a collagenous domain, and a fibrinogen-like domain at the C-terminus (Matsushita et al. 1996, Lu and Le 1998). Three polypeptide chains oligomerize through the collagenous region to form the basic structural subunit, a triple helix. L-ficolin circulates in serum as a tetramer of this structural subunit, thus having 12 identical polypeptide chains (Hummeslshoj et al. 2007).

Complement activation: The fibrinogen-like domain binds to ligands such as acetylated sugar residues (N-acetylg glucosamine, N-acetylg glucosaminic acid, NeuNAc) and 1,3 β-D glycan on a variety of pathogens (Garlatti et al. 2007, Krarup et al. 2004, Ma et al. 2004) (see 'Interactions With Ligands and Other Proteins' section). The triple helix formed by the collagenous regions is bound to serine proteases such as MASP-1 and MASP-2 (Gaboriaud et al. 2007). Upon binding to pathogen surfaces, MASP-1 and MASP-2 get activated (through proteolysis), which in turn sequentially cleave complement proteins C4 and C2 (Héja et al. 2012a, Héja et al. 2012b). Thus, the lectin pathway of complement gets activated leading to complement attack on pathogens. Even though, L-ficolin is structurally similar to other collectins, it has unique pathogenic ligands making it an important player in host resistance against pathogens (Krarup et al. 2005).

Opsonophagocytosis: C4 and C2 cleaved by activated L-ficolin-MASP complex form C3-convertase (C4b2a). C3b generated by action of C3-convertase on C3, acts as an opsonin when deposited on pathogen surfaces. L-ficolin can bind calreticulin (CRT,cC1qR) (Lacroix et al. 2009), which may aid ficolin-mediated phagocytosis of pathogens such as Salmonella (Matsushita et al. 1996), group B streptococci (Aoyagi et al. 2005) and Pseudomonas aeruginosa (Zhang et al. 2009). Apart from phagocytosis of pathogens, L-ficolin can also aid in clearance of late apoptotic and necrotic host cells (Kuraya et al. 2005, Jensen et al. 2007).

Activation of coagulation system: Active MASP-1 and MASP-2 have been shown to act on coagulation proteins. MASP-1 has thrombin-like activity while MASP-2 has been shown to generate thrombin from prothrombin (Krarup et al. 2007, Presanis et al. 2004). A more direct evidence of L-ficolin bound MASP complex form C3-convertase (C4b2a). C3b generated by action of C3-convertase on C3, acts as an opsonin when deposited on pathogen surfaces. L-ficolin can bind calreticulin (CRT,cC1qR) (Lacroix et al. 2009), which may aid ficolin-mediated phagocytosis of pathogens such as Salmonella (Matsushita et al. 1996), group B streptococci (Aoyagi et al. 2005) and Pseudomonas aeruginosa (Zhang et al. 2009). Apart from phagocytosis of pathogens, L-ficolin can also aid in clearance of late apoptotic and necrotic host cells (Kuraya et al. 2005, Jensen et al. 2007).

REGULATION OF ACTIVITY
The activity of L-ficolin can be primarily regulated at the following levels: serum concentration, efficacy of binding to pathogens and during cleavage of MASP proteins (Skjoedt et al. 2010). Serum concentration of L-ficolin is weakly regulated by
the presence of polymorphisms (see 'Regulation of Concentration' section). Binding to pathogens is also regulated by polymorphisms (see 'Phenotypes' section), capsulation of pathogens such as group B streptococci (Aoyagi et al. 2008, Krarup et al. 2005) or by presence of adaptor proteins (see 'Interactions With Ligands and Other Proteins' section). Apart from these factors, karilysin, a matrix metalloproteinase-like enzyme produced by periodontal pathogen Tannerella forsythia, cleaves L-ficolin along with other complement proteins, thereby inhibiting complement activation (Jusko et al. 2012).

INTERACTIONS

Twelve polypeptide chains of L-ficolin (as explained in 'Protein Function' section) oligomerize to form a functional complex. L-ficolin interacts with several host and pathogenic factors:

L-ficolin-MASP Complex: Similar to M-ficolin and H-ficolin, the tetrameric form of L-ficolin is in complex with different MASP proteins via its collagen region (Lacroix et al. 2009, Gabariaud et al. 2007), as listed below. All the MASP proteins bound to L-ficolin are in homo-dimeric form (Gregory et al. 2004, Teillet et al. 2008) and the binding is dependent on physiological concentrations of calcium (Cseh et al. 2002). MASP-1 and MASP-2 encoded by MASP1 and MASP2 genes respectively, are serine proteases (Matsushita et al. 2000, Matsushita and Fujita 1992, Thiel et al. 1997). MASP-1 is auto-activated, when in complex with L-ficolin bound to acetylated sugar residues on pathogen surfaces. Activated MASP-1 cleaves and activates MASP-2 (Héja et al. 2012a, Héja et al. 2012b, Degn et al. 2012). MASP-2 sequentially activates C4 and C2 through its serine protease activity. MASP-3 is a splice variant of MASP1 gene and binds to L-ficolin (Dahl et al. 2001, Teillet et al. 2008). It has a serine protease domain, but has no known physiological relevant substrates. In mice however, MASP-3 has been shown to be important in alternative pathway of complement activation (Iwaki et al. 2011) whereas in humans the presence of MASP-3 is not essential for the alternative pathway (Degn et al. 2012). MASP-3 has been shown to compete with MASP-2 to bind to MBL, resulting in down-regulation of lectin pathway activation (Dahl et al. 2001). However, the physiological role of MASP-3 binding to L-ficolin is yet to be determined. Map44, expressed mainly in the heart, is yet another splice variant of MASP1 gene. It however does not have a serine protease domain and is found in complex with L-ficolin (Skjoedt et al. 2011, Skjoedt et al. 2010, Degn et al. 2009). sMAP (MaP19), a splice variant of MASP2 gene, also lacks a serine protease domain and binds to L-ficolin (Cseh et al. 2002, Gregory et al. 2004). Further, MAP44 and sMAP are both able to bind to the parts of the helix formed by the collagen-like regions of MBL and thus compete with MASP-2 for binding and thereby down-regulating complement activation (Degn et al. 2009, Iwaki et al. 2006). It is therefore very likely that MAP44 and sMAP play a similar role in their interaction with L-ficolin, but it is yet to be demonstrated.

Interactions with other host factors: L-ficolin can bind to chaperones such as calreticulin and to other recognition molecules, e.g. C-reactive protein (CRP) and pentraxin-3 (PTX3), and all of these interactions enhance complement activation (Lacroix et al. 2009, Zhang et al. 2009, Ma et al. 2009) and thereby increase resistance against pathogens. Binding to CRT or DNA from apoptotic cells also aids clearance of late apoptotic and necrotic cells (Kuraya et al. 2005, Jensen et al. 2007). However, L-ficolin can also bind to artificially acetylated low-density lipoprotein (ac-LDL) and activate the lectin pathway (Faro et al. 2008).

Interactions with pathogens: L-ficolin via its fibrinogen-like domain binds to several pathogen surfaces, including 1,3β-D glycan. It primarily recognizes acetylated sugar residues on different gram-positive and gram-negative bacteria (Krarup et al. 2004, Krarup et al. 2008), including GlcNAc residues on Salmonella typhimurium (Taira et al. 2000) and Streptococcus pneumoniae (Krarup et al. 2004). L-ficolin binds to NeuGcNAc residues on capsulated forms of group B streptococci (GBS), while MBL failed to bind to these capsulated pathogens (Aoyagi et al. 2005, Aoyagi et al. 2008), implying an important role for L-ficolin in clearance of capsulated pathogens. In fact, L-ficolin exclusively bound to capsulated forms of Staphylococcus aureus and Streptococcus pneumoniae (Krarup et al. 2005). Lipoteichoic acid (LTA), found on cell wall of gram-positive bacteria, can bind to L-ficolin (Lynch et al. 2004). Escherichia coli (Hummelshøj et al. 2012) and Mycobacterium bovis BCG (Carroll et al. 2009) can bind to L-ficolin. Viruses, such as hepatitis C (HCV) and influenza A are bound to L-ficolin. N-glycans of HCV envelope glycoproteins (Liu et al. 2009) and glycoproteins hemagglutinin and neuraminadase of influenza A (Pan et al. 2012) are L-ficolin ligands. L-ficolin also binds to the surfaces of Trypanosoma cruzi (pathogen causing Chagas disease) (Cestari et al. 2009, Cestari et al. 2010) and Giardia intestinalis (Evans-Osses et al. 2010), and likely aids in clearance of these pathogens.

CMAP, a complement database, documents the biochemical methods used to identify these interactions (Yang et al. 2013).

PHENOTYPES

L-ficolin is produced by expression of ficocin-2 (FCN2) gene, which is located on chromosome 9q34 and consists of eight exons (Endo et al. 1996). A large number of single nucleotide polymorphisms (SNPs) are found in both the promoter and coding regions across varied ethnic groups (Herpers et al. 2006, Hummelshøj et al. 2008). The most common SNPs in Caucasians occur at the following positions: -986, -602, -557, -64, -4 in the promoter region and +2488, +6359, +6424 in the exon region (Herpers et al. 2006, Hummelshøj et al. 2005). While generally polymorphisms in the promoter and coding regions affect serum concentration and pathogen-binding efficacy respectively, polymorphism at +6424 position (from ATG site) is known to affect both serum levels and binding efficacy (Munthe-Fog et al. 2007, Hummelshøj et al. 2005). Several studies report association of lower serum levels of L-ficolin (resulting from SNPs) with recurrent respiratory tract infections (RTI) in children (Atkinson et al. 2004, Cedzynski et al. 2009, Cedzynski et al. 2007) and also with pre-mature birth and lower birth weight in Polish neonates (Sweirzko et al. 2009). However, a recent study has shown no associations between genotypes and lower birth weight or perinatal infections (Kilpatrick et al. 2013). Lower serum levels are also associated with schistosomiasis (Ouf et al. 2012), streptococcal infection (Messias-Reason et al. 2009) and bronchiectasis (Kilpatrick et al. 2009), chronic Chagas disease (Luz et al. 2013) and inversely correlated with hereditary angioedema due to C1-inhibitor deficiency (HAE-C1-INH) (Csuka et al. 2013). SNP causing Thr236Met substitution at the fibrinogen-like residues on capsulated forms of group B streptococci (GBS), while MBL failed to bind to these capsulated pathogens (Aoyagi et al. 2005, Aoyagi et al. 2008), implying an important role for L-ficolin in clearance of capsulated pathogens. In fact, L-ficolin exclusively bound to capsulated forms of Staphylococcus aureus and Streptococcus pneumoniae (Krarup et al. 2005). L-ficolin via its fibrinogen-like domain binds to several pathogen surfaces, including 1,3β-D glycan. It primarily recognizes acetylated sugar residues on different gram-positive and gram-negative bacteria (Krarup et al. 2004, Krarup et al. 2008), including GlcNAc residues on Salmonella typhimurium (Taira et al. 2000) and Streptococcus pneumoniae (Krarup et al. 2004). L-ficolin binds to NeuGcNAc residues on capsulated forms of group B streptococci (GBS), while MBL failed to bind to these capsulated pathogens (Aoyagi et al. 2005, Aoyagi et al. 2008), implying an important role for L-ficolin in clearance of capsulated pathogens. In fact, L-ficolin exclusively bound to capsulated forms of Staphylococcus aureus and Streptococcus pneumoniae (Krarup et al. 2005). Lipoteichoic acid (LTA), found on cell wall of gram-positive bacteria, can bind to L-ficolin (Lynch et al. 2004). Escherichia coli (Hummelshøj et al. 2012) and Mycobacterium bovis BCG (Carroll et al. 2009) can bind to L-ficolin. Viruses, such as hepatitis C (HCV) and influenza A are bound to L-ficolin. N-glycans of HCV envelope glycoproteins (Liu et al. 2009) and glycoproteins hemagglutinin and neuraminadase of influenza A (Pan et al. 2012) are L-ficolin ligands. L-ficolin also binds to the surfaces of Trypanosoma cruzi (pathogen causing Chagas disease) (Cestari et al. 2009, Cestari et al. 2010) and Giardia intestinalis (Evans-Osses et al. 2010), and likely aids in clearance of these pathogens.

CMAP, a complement database, documents the biochemical methods used to identify these interactions (Yang et al. 2013).
contrast, SNPs known to cause low serum levels or decreased binding are not associated with pneumococcal disease (Chapman et al. 2007). Presence of Ala258Ser variant of L-ficolin in the donor predicts an improved renal transplant outcome, which may be attributed to increased binding to GlcNAc (Eikmans et al. 2013). Haplotypes which produce normal levels of L-ficolin offer protection against leprosy (de Messias-Reason et al. 2009) while certain ficolin-2 genotypes are associated with leprosy in a specific Chinese population (Zhang et al. 2013). Higher levels of L-ficolin due to AGACG (at positions -986, -602, -4, +6359 and +6424) and AAAG (at positions -986, -602, -4, and +6424) haplotypes adversely affect patients with leishmaniasis and hepatitis B infection respectively (Assaf et al. 2012, Hoang et al. 2011). L-ficolin can also adversely affect host tissue during nephropathy (Roos et al. 2006).

MAJOR SITES OF EXPRESSION
L-ficolin is produced by the hepatocytes in liver (Matsushita et al. 1996).

SPLICE VARIANTS
L-ficolin, located on chromosome 9q34 consists of eight exons and has no known splice variants (Endo et al. 1996).

REGULATION OF CONCENTRATION
Serum levels of L-ficolin have been measured in a number of studies involving a large number of subjects. These studies report median values of L-ficolin in normal healthy individuals to be 4.13 μg/ml, 3.7 μg/ml, 3.3 μg/ml and 3.0 μg/ml respectively (Le et al. 1998, Kilpatrick et al. 1999, Krarup et al. 2005, Gulla et al. 2006). Almost all of these studies used ELISA for measurement (Taira et al. 2000). Age and gender did not affect serum levels in adults (Kilpatrick et al. 1999). However, cord blood samples show much lower levels, with a median of 2.5 μg/ml (Le et al. 1998, Kilpatrick et al. 1999). Sallenbach et al. (2011) reported the levels from various age groups ranging from neonates (2.8 μg/ml), 1> yr (7.08 μg/ml), 1-4 yr (11.3 μg/ml), 4-16 yr (8.66 μg/ml) and adults (3.37 μg/ml). Three polymorphisms in the promoter region were shown to affect serum levels by two-fold increase or decrease (Munthe-Fog et al. 2007, Hummelshoj et al. 2005). While generally polymorphisms in exon regions have been attributed to binding efficiency, one polymorphism in +6424 position (from ATG start site) results is lower serum concentration (Munthe-Fog et al. 2007). Serum levels of L-ficolin are known to increase during acute phase of malaria (Faik et al. 2011). Several diseases have been associated with lower serum concentration, which are further discussed in 'phenotypes' section.

ANTIBODIES
Table 1: Functional States

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<th>STATE DESCRIPTION</th>
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<td>L-FCN (native)</td>
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<td>extracellular region</td>
<td>Cseh S et al. 2002; Lacroix M et al. 2009</td>
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<td>L-FCN/MASP-2</td>
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<tr>
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ACKNOWLEDGEMENTS

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SUPPLEMENTARY

Supplementary information is available online.

REFERENCES


Gulla KC, Gupta K, Krarup A, Gal P, Schwaebel WJ, Sim RB,


This molecule exists in 23 states, has 25 transitions between these states and has 2 enzyme functions. (Please zoom in the pdf file to view details.)
The TSH receptor is a member of the G protein-coupled receptor (GPCR) family. It is one of the glycoprotein hormone receptors, which also includes the FSH and LH/CG receptors. The TSH receptor mediates the action of the pituitary-derived glycoprotein, TSH (thyroid stimulating hormone, thyrotropin or thyrotrophin). TSH binds to the TSH receptor which is located on thyroid follicular cells (but is also expressed in extrathyroidal sites). Glycosylation of the TSH receptor occurs, as does cleavage of the receptor from an intact to an extracellular form (α subunit), which may be shed after deletion of a short region (aa 316-366) near the C terminal of the extracellular domain, thus leaving a transmembrane form (β subunit). The α subunit is responsible for ligand/autoantibody binding, facilitated by glycosylation and possibly by the extracellular loops of the 7 transmembrane segments. The intracellular loops of the β subunit interact with G proteins when the receptor is activated. The receptor may also exist in multimeric forms, although it is not clear whether these forms play a role in TSH receptor function. TSH action involves cAMP and IP/DAG responses. The TSH receptor controls positively both the function (production of thyroid hormones T3 and T4) and growth of the thyroid.

KEYWORDS
Thyroid stimulating hormone receptor; Thyrotropin receptor; Thyrotropin receptor; Tsh receptor; TSH-R; Tshr

IDENTIFIERS
Molecule Page ID:A002333, Species:Mouse, NCBI Gene ID: 22095, Protein Accession:NP_035778.3, Gene Symbol:Tshr

PROTEIN FUNCTION
The TSH (thyroid stimulating hormone, thyrotropin or thyrotrophin) receptor is a seven transmembrane domain receptor which mediates the action of the pituitary derived glycoprotein TSH. TSH binds to the TSH receptor located on the basolateral surface of thyroid follicular cells and facilitates production of the thyroid hormones T4 and T3. The TSH receptor is a member of the G protein-coupled receptor superfamily. It is proposed that the basal state of the receptor is due to inhibition of function resulting from interaction between the extracellular and transmembrane domains; thus the extracellular domain itself functions as a tethered inverse agonist (Vlaeminck-Guilleme et al. 2002). Binding of TSH to the extracellular domain is proposed to lead to full activation of the receptor. In addition to stimulation of thyroid hormone production, TSH stimulates iodine uptake, thyroid growth and also protects thyroid cells from apoptosis (Szkudlinski et al. 2002). The TSH receptor is activated by TSH, thyroid autoantibodies and activating mutations (Rodien et al. 2003, Kleinau et al. 2007). Outside the autoimmune field, abnormal function of the TSH receptor is associated with congenital hypothyroidism, hereditary nonautoimmune hyperthyroidism and toxic adenomas(Schoneberg 2004, Szkudlinski et al. 2002, Corvilain et al. 2001, Gutierrez et al. 2004, Smit et al. 2007, Vassart and Costagliola. 2011).

REGULATION OF ACTIVITY
Binding of TSH occurs to specific residues in the concave portion of the extracellular leucine-rich repeat domain (LRRD) of the TSH receptor (Nagayama et al. 1990, Smits et al. 2003). After bovine TSH binding to the human TSH receptor, interaction with the so-called “hinge” region, common to other glycoprotein hormone receptors, occurs within the N-terminal extracellular region. Thereafter, conformational changes in the receptor and signal transduction through the transmembrane region occur (Mueller et al. 2008, Vassart et al. 2009). Glycosylation of the TSH receptor occurs, as does cleavage of the receptor from an intact to an extracellular form (α subunit) (which may be shed) and a transmembrane form (β subunit), which is membrane bound (Rapoport et al. 1998). The intracellular loops of the β subunit interact with G-proteins (Gαs and Gαi; Allgeier et al. 1994) when the receptor is activated. The receptor may also exist in multimeric forms although the role in TSH receptor function is not clear. TSH activation leads to cAMP and inositolphosphate responses (Van Sande et al. 1995).

INTERACTIONS
The extracellular region of the TSH receptor interacts with its ligand TSH (reviewed in Rapoport et al. 1998 and Szkudlinski et al. 2002). G protein recognition occurs upon the intracellular loops of the receptor (Claus et al. 2006). Although a high degree of sequence homology exists with other glycoprotein hormone receptors, this is not usually of any clinical consequence. Exceptions are rare conditions such as hydatidiform mole, choriovitamnoma, metastatic embryonal carcinoma of the testis and mutations of the hormone binding domain of the receptor, in which HCG cross-reacts with the TSH receptor and induces hyperthyroidism (Pekonen et al. 1988, Hershman et al. 1988, Yoshikawa et al. 1989, Rodien et al. 1998).

Two glycoprotein hormone subunits (glycoprotein hormone α2-subunit or GPA2 and glycoprotein hormone β5-subunit or GPB5), localised in pituitary, eye or testis in the rat, and known as thyrostimulin, form heterodimers in vitro and show affinity for the TSH receptor, similar to that seen with TSH (Nagasaki et al. 2006).

In addition to the endogenous ligand TSH, TSH receptor stimulating autoantibodies (associated with Graves’ disease) bind to the TSH receptor, typically to epitopes of the extracellular component of the receptor which are distinct (allosteric) from binding sites for TSH (ie., orthosteric binding sites) (Sanders et al. 2007, Costagliola et al. 2004). A number of TSH receptor monoclonal antibodies that bind to various allosteric sites of the receptor and may also act as agonists,
antagonists or inverse agonists have been described (Sanderson et al. 2004, Chen et al. 2008, Chen et al. 2009, Sanderson et al. 2010). Small molecule ligands of the TSH receptor, which have agonistic or antagonistic activity to TSH and TSH stimulating antibodies have also been developed (Neumann et al. 2008, Neumann et al. 2009, Neumann et al. 2011), as have modified forms of TSH, which may possess properties of superagonism (Szkudlinski et al. 1996, Grossmann et al. 1998, Leitolf et al. 2000) or antagonism (Morris et al. 1990, Fares et al. 2001).

PHENOTYPES
TSH receptor knockout mice present with developmental and growth delays and are profoundly hypothyroid, dying within one week of non-replacement with thyroid hormone (Marianis et al. 2002) although heterozygotes are unaffected. Affected animals produce uniodinated thyroglobulin and lack iodine-iodide symporter expression. An immune response to the TSH receptor is inducible in TSH receptor knockout mice (Nakahara et al. 2010).

Selective knockout of the TSH receptor in adipocytes using the Cre-loxP system results in reduced TSH-induced lipolysis (Elgadi et al. 2010).

GPB5 knockout mice show hypothyroxinemia, especially in juvenile mice (van Zeijl et al. 2010).

Selective knockout of the α subunit of Gαi3 in the thyroid in mice results in reduced iodine organification and thyroid hormone secretion in response to TSH, hypothyroidism and a lack of thyroid proliferative response to TSH or a goitrogenic diet (Kero et al. 2007).


MAJOR SITES OF EXPRESSION
The major site of TSH receptor expression is the thyroid follicular cell, but extrathyroidal expression also occurs. The TSH receptor protein has been detected in fibroblasts and adipose tissue in normal subjects and in patients with Graves’ ophthalmopathy and pretibial myxoedema and is involved in adipogenesis (Daumiere et al. 2002). In addition, the TSH receptor has been detected in extraocular, but not non-ocular or cardiac muscle of normal subjects (Kloprogge et al. 2005, Kloprogge et al. 2006, Busuttil et al. 2001). It is also present in the brain in ependymal cells of the mediobasal hypothalamus, where it participates in the photoperiodic regulation of endocrine functions (Ono et al. 2008, Yasuo et al. 2011). TSH receptor transcripts are also found in other extrathyroidal sites (in the mouse): these include the olfactory bulb, pituitary gland, retina, adrenal gland, aorta, vena cava, trachea, kidney, bone marrow, ovary, testis, uterus and skin (Regard et al. 2008; data are available at http://pdp.med.unc.edu/ShaunCell/home.php). The functional significance of these extrathyroidal TSH receptor transcripts is unknown. The TSH receptor is also present in osteoclasts and may have an anti-resorptive role in bone function (Abe et al. 2003, Abe et al. 2007, Sun et al. 2008). However, others have found that thyroid hormone, rather than TSH, may play a role in regulating bone function (Bassett et al. 2008).

splice variants
The major transcript encoding the human TSH receptor has 10 exons and a transcript length of 4410 bp (found at http://www.ncbi.nlm.nih.gov/nucleotide/NM_000369.2). Two alternatively spliced variants, "transcript variant 2" and "transcript variant 3", are described (found at http://www.ncbi.nlm.nih.gov/nucleotide/NM_001018036.2 and NM_001142626.2, respectively). There are a number of other splice variants described (found at http://ncbi.nlm.nih.gov/IEB/Research/Assembly/av.cgi?c=geneid &org=9606&l=7253). There is no evidence for any functional relevance of these splice variants.

The TSH receptor is also subject to post-translational cleavage. Thus, a short polypeptide region (aa 316-366) near the C terminal of the extracellular domain may be deleted (Loosfelt et al. 1992, Chazenbalk et al. 1997), leading to a subunit loss (Couet et al. 1996). The functional relevance of this α subunit shedding is unclear, but it may play a role in receptor signalling, trafficking and internalization (Latif et al. 2009).

REGULATION OF CONCENTRATION
The gene encoding the TSH receptor is localised on chromosome 14q31 and consists of 10 exons, over 60kb (Libert et al. 1990, Rousseau-Merck et al. 1990, Gross et al. 1991). Downregulation of the TSH receptor protein occurs in the presence of its natural ligand TSH (Nagayama et al. 1994). In addition, phosphodiesterase induction downregulates cAMP accumulation when the receptor is stimulated (Persani et al. 2000). Subsequent recycling to the cell surface occurs (Baratti-Elbaz et al. 1999) and involves an hScrip-betaPIX-GIT1-ARF6 pathway (Lahuna et al. 2005).

ANTIBODIES
Anti-TSH receptor antibodies are commercially available.

Abnova
Mouse monoclonal antibody clone 4C1/E1/G8 against extracellular region of recombinant human TSH receptor (catalog number MAB3576).

Mouse monoclonal antibody clone 3A6 against full length recombinant human TSH receptor (catalog number MAB6678).

Rabbit polyclonal antibody against synthetic peptide of extracellular region of human TSH receptor (catalog number PAB15551).

ThermoScientific
Mouse monoclonal antibody clone 28 against extracellular region of recombinant human TSH receptor (catalog number MA3-217).

Mouse monoclonal antibody clone 49 against extracellular region of recombinant human TSH receptor (catalog number MA3-218).

Rabbit polyclonal antibody against synthetic peptides of human
TSH receptor conjugated to KLH (catalog number PA1-23530).

**RSR Limited for Autoimmune Diagnostics**

Monoclonal antibody against human TSH receptor (blocking type)(catalog number K170/FD/0.01).

Monoclonal antibody against human TSH receptor (stimulating type)(catalog number M22/FD/0.004 and M22/FD/0.04).
Table 1: Functional States

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REFERENCES


