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Leukocyte surface antigen CD47

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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/cGMP 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

IDENTIFIERS

Molecule Page ID:A005186, Species:Human, NCBI Gene ID: 961, Protein Accession:NP_001768.1, Gene Symbol:CD47

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 carboxy-terminal 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 substrate-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

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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). Signaling 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 (Sarfati *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 SIRPa 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, K-[1016]RFYVVMWK[1024]-K) 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 Th1 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, [1102]FIRVVMYEGKK[1112]). 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 Ser[64] (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 $\alpha_v\beta_3$, $\alpha_{IIb}\beta_3$, $\alpha_2\beta_1$ and $\alpha_4\beta_1$ (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 $\alpha_v\beta_3$ integrin but directly activates $\alpha_4\beta_1$ 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 $\alpha_2\beta_1$ (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 ($\Psi\Delta m$) (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-immunoprecipitaton 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 and the nuclear encoded transcription factor PGC1α and its downstream effector NRF1. PGC1α is a major transcription factor for controlling mitochondrial biogenesis, suggesting that CD47 is a negative regulator of mitochondrial biogenesis. Mitochondrial function may also be regulated by CD47 because aortic smooth muscle cells and isolated mitochondria from CD47 null mice exhibited less mitochondrial ROS production, while the mice exhibited lower oxygen consumption that WT mice. Consistent with increased mitochondrial efficiency and capacity, the null mice exhibited increased exercise endurance on a treadmill running protocol. These phenotypes diminished as the mice aged. NO/cGMP, Drp1, autophagy, and cAMP pathways are known to regulate mitochondrial biogenesis, but it is not known which of these CD47-dependent pathways account for the selective increase in mitochondria levels in CD47 null muscle.

Other CD47 signaling targets

TSP1 peptides that bind to CD47 have several other demonstrated activities for which specific signaling pathways have not been established, including inducing histamine release from mast cells (Sick *et al.* 2009), increasing adhesion of RBC from patients with sickle cell disease (Brittain *et al.* 2001), inhibiting development of human naive T cells into Th1 effectors (Avice *et al.* 2001), inhibiting FGF-2-stimulated angiogenic responses in vitro and in vivo (Kanda *et al.* 1999), and downregulation of pro-inflammatory interleukin (IL)-12 and tumor necrosis factor-α production by monocyte-derived dendritic cells (Demeure *et al.* 2000; Johansson and Londei 2004).

Interpretation of TSP1 peptide data

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 stimulate aggregation and tyrosine phosphorylation in platelets from CD47-null mice (Tulasne *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 chrondroitin 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 Ser[64] and Ser[79] (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 Ser[64].

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 $\alpha_6\beta_1$ 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 $\alpha_v\beta_3$, 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 $\alpha_{IIb}\beta_3$ form a complex with the tyrosine kinases Src, FAK and Syk (Chung *et al.* 1997). Furthermore, CD47 and integrin $\alpha_V\beta_3$ 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 identified 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 adenylate 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 platlets (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 $\alpha_4\beta_1$ 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 myocutaneous 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

Osteoclast formation stimulated by human myeloma cells was limited by agents that suppress activation of CD47 (Kukreja *et al.* 2009).CD47-null mice display decreased osteoclast activity in vitro, reduced osteoclast numbers in bone in vivo and increased bone mass (Lundberg *et al.* 2007; Uluçkan *et al.* 2009). In cultured normal and diseased condrocytes a CD47 antibody decreased proteoglycan production (Holledge *et al.* 2008).

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 a 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 (inlcuding NO) and over-active vasoconstrictor responses. Analysis of lung parenchyma from PAH pateints has found signficant 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 endogneous 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 (Isenberg *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 upregualted 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 astrocites 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 stimualted signaling occurred through CD47 and several other cell surface protiens (Bamberger *et al*. 2003). These findings are made more relavent 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 (Oldenborg *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

(Ticchioni *et al*. 1997;Wilson *et al*. 1999;Li *et al*. 2001). This costimulation involves activation of Ras, JNK, ERK1, Elk1, and AP1 and is mediated in part by CD47 functioning as an integrin activator to promote T cell spreading (Wilson *et al*. 1999;Reinhold *et al*. 1997; Reinhold *et al*. 1999). In contrast to these costimulatory activities, CD47 ligation by TSP1 inhibits CD3-dependent T cell activation independent of β1 integrins and requiring expression and glycosaminoglycan modification of CD47 (Li *et al*. 2001; Li *et al*. 2002; Kaur *et al*. 2011). CD47 on antigen presenting cells (APC) may play an additional stimulatory role in T cell activation. Binding of CD47 expressed on APC to SIRPα on T cells results in enhanced antigen-specific T cell proliferation (Piccio *et al*. 2005). T cell activation also requires autocrine signaling by the gasotransmitter hydrogen sulfide (Miller *et al*. 2012). 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).

CD4+ T cells differentiate into several lineages in response to specific environmental cytokines and signals from APC (O'Shea *et al*. 2010). CD47 regulates this process on both APC and T cells. CD47-Fc inhibits IL-12, TNF-α, IL-6, and IL-10 production by immature dendritic cells primed with endotoxin,thereby inhibiting their functional maturation (Latour *et al*. 2001). Ligation of SIRPα or CD47 also decreased anti-CD3-activated T cell IL-12 responsiveness as measured by IFN-γ production. CD47 is a negative regulator of T cell differentiation along the Th1 lineage (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 (Bouguermouh *et al*. 2008). This bias is consistent with the exaggerated contact hypersensitivity response in CD47 deficient mice (Bouguermouh *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 reprograming 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 cells results from their elevated expression of c-Myc, Sox2, Oct4, and Klf4. Similarly increased expression of stem cell transcription factors was found in some tissues of CD47-null mice and in human T cells lacking CD47. Ligation of CD47 by TSP1 decreased c-Myc expression in WT human T cells, and the inhibitory effects of re-expressing CD47 in CD47-deficient cells could be overcome by elevating expression of c-Myc. Thus, CD47 negatively regulates stem cell self-renewal by limiting the expression of c-Myc and other stem cell transcription factors.

MAJOR SITES OF EXPRESSION
CD47 is a broadly expressed transmembrane protein. Widespread expression of CD47 is believed to be important for innate immune cells to recognize between foreign and self.

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.

Form 2 appears to be the most widely expressed and is the dominant form found in all circulating and immune cells in both mouse and human. In humans, little information is available on the tissue distribution of CD47 isoform expression. However, in mouse, a detailed analysis of normal tissues was performed. Forms 1 and 3 were only weakly expressed, whereas form 4, the longest, was found to predominate in brain, intestine and testis (Reinhold *et al*. 1995). In the rat hippocampus, IAP5 and IAP6 mRNA expressions, but not that of IAP7 were increased in response to training, implying a role of the former splice isoforms in memory consolidation (Lee *et al*. 2000). IAP7 was the predominant form is astrocytes.

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).

REGULATION OF CONCENTRATION
Promoter and transcriptional regulation

A core promoter for the CD47 gene was localized between nucleotide positions −232 and −12 relative to the translation 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).

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 (Junker *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 effectors 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 increase apoptosis due to faulty CD47 signaling (Catani *et al*. 2011)

Hemophagocytic lymphohistiocytosis is an autosomal recessive disorder that is associated with parental 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 blood (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 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-I 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 (Rendtlew 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, brain, 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 cytolysis 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 cytotoxicity 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

1/1A4 (flow cytometry)

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 αvβ3 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

Sources: Santa Cruz, Biolegend, Abcam

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

Sources: Santa Cruz, Biolegend, Abcam

MEM122

Mouse IgM reacts with human, primate, porcine CD47

Table 1: Functional States

STATE DESCRIPTION	LOCATION	REFERENCES
CD47	Unknown	
CD47-N16,55,93G3	ER body	Parthasarathy R *et al.* 2006; Mawby WJ *et al.* 1994
C47 PR (mature)	ER body	Mawby WJ *et al.* 1994
CD47-pyroGlu (blocked)	ER body	Mawby WJ *et al.* 1994
CD47 pyroGlu (cell surface)	cell surface	Mawby WJ *et al.* 1994; Miller YE *et al.* 1987; Parthasarathy R *et al.* 2006
CD47/SIRPα	cell surface	Matozaki T *et al.* 2009; Subramanian S *et al.* 2006; Hatherley D *et al.* 2008
CD47/SIRPβ	cell surface	Hatherley D *et al.* 2008
CD47/SIRPγ	cell surface	Brooke G *et al.* 2004
CD47/TSP1	cell surface	Isenberg JS *et al.* 2009; Kaur S *et al.* 2011
CD47/TSP2	cell surface	Isenberg JS *et al.* 2009
CD47/TSP4	cell surface	Isenberg JS *et al.* 2009
CD47/CD47	cell surface	Rebres RA *et al.* 2005
CD47/αVβ3	cell surface	Lindberg FP *et al.* 1996; Lindberg FP *et al.* 1993
CD47/αIIbβ3	cell surface	Chung J *et al.* 1997
CD47/αIIbβ3/FAK	cell surface	Chung J *et al.* 1997
CD47/αIIbβ3/Syk	cell surface	Chung J *et al.* 1997
CD47/αVβ3/Lyn	cell surface	McDonald JF *et al.* 2004
CD47/αVβ3/SHP2	cell surface	McDonald JF *et al.* 2004
CD47/αVβ3/Src	cell surface	McDonald JF *et al.* 2004
CD47/αIIbβ3/Src	cell surface	Chung J *et al.* 1997
CD47/VEGFR2	cell surface	Kaur S *et al.* 2010
CD47/αVβ3/Gαi2-GDP/βγ	cell surface	Frazier WA *et al.* 1999
CD47/αVβ3/Gαi2-GTP/βγ	cell surface	Frazier WA *et al.* 1999
CD47/αVβ3/Gαi2-GTP	cell surface	Frazier WA *et al.* 1999
CD47/BNIP3	cell surface	Lamy L *et al.* 2003
CD47/Rh complex	cell surface	Dahl KN *et al.* 2003
CD47-PR (cleaved extracellular domain)	Unknown	Kaur S *et al.* 2011; Maile LA *et al.*
CD47-heparan sulfate	cell surface	Kaur S *et al.* 2011
CD47/PLIC	cell surface	N'Diaye EN and Brown EJ 2003
CD47-chondroitin sulfate	cell surface	Kaur S *et al.* 2011

SUPPLEMENTARY

Supplementary information is available online.

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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.)