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The Matricellular Protein Thrombospondin-1 Globally Regulates Cardiovascular Function and Responses to Stress via CD47

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Abstract

Matricellular proteins play diverse roles in modulating cell behavior by engaging specific cell surface receptors and interacting with extracellular matrix proteins, secreted enzymes, and growth factors. Studies of such interactions involving thrombospondin-1 have revealed several physiological functions and roles in the pathogenesis of injury responses and cancer, but the relatively mild phenotypes of mice lacking thrombospondin-1 suggested that thrombospondin-1 would not be a central player that could be exploited therapeutically. Recent research focusing on signaling through its receptor CD47, however, has uncovered more critical roles for thrombospondin-1 in acute regulation of cardiovascular dynamics, hemostasis, immunity, and mitochondrial homeostasis. Several of these functions are mediated by potent and redundant inhibition of the canonical nitric oxide pathway. Conversely, elevated tissue thrombospondin-1 levels in major chronic diseases of aging may account for the deficient nitric oxide signaling that characterizes these diseases, and experimental therapeutics targeting CD47 show promise for treating such chronic diseases as well as acute stress conditions that are associated with elevated thrombospondin-1 expression.

Keywords

Thrombospondin-1; nitric oxide; blood pressure; ischemia; CD47; cGMP

1. Introduction

Thrombospondin-1 (TSP1) is a matricellular protein that is transiently expressed in extracellular matrix and modulates cell function in a context-specific manner by engaging cell surface receptors and other components of the extracellular matrix (Bornstein, 1995; Roberts and Lau, 2011). TSP1 circulates at ~100 pM levels in plasma, and platelet α -

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Disclosures

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granules represent a major preformed storage pool that can rapidly increase extracellular concentrations of the protein at sites of injury. Studies reviewed here comparing vascular responses of wild type (WT) and TSP1 null mice show that endogenous levels of TSP1 in vascular tissues and circulating in plasma serve important physiological and pathophysiological functions.

1.1. TSP1 and the CD47 receptor on vascular cells

Several excellent reviews broadly describe the structure and interactions of TSP1 (Carlson et al., 2008; Elzie and Murphy-Ullrich, 2004; Mosher and Adams, 2012; Murphy-Ullrich and Iozzo, 2012; Risher and Eroglu, 2012; Sweetwyne and Murphy-Ullrich, 2012; Tan and Lawler, 2009; Murphy-Ullrich and Iozzo, 2012), so we focus here on its interactions with the receptor CD47. CD47 is an integral membrane protein containing an extracellular IgV domain, a domain that spans the membrane 5 times that is probably derived from a presenilin transmembrane domain (Watanabe et al., 2010), and a short variably spliced C-terminal cytoplasmic tail (Frazier et al., 2010) (Fig. 1). CD47 is universally expressed by vascular and circulating blood cells of land-dwelling vertebrates.

TSP1 engages CD47 via its C-terminal domain (Frazier et al., 2010). CD47 was originally identified as a TSP1 receptor based on its binding to an immobilized peptide derived from this domain of TSP1 that contains a VVM motif (Gao et al., 1996). Although this motif is conserved in other mammalian TSPs, subsequent studies showed CD47 binding to be relatively specific for TSP1 (Isenberg et al., 2009a). Furthermore, the two VVM motifs in the C-terminal domain of TSP1 are not exposed to solvent (Kvansakul et al., 2004). Thus, the residues on TSP1 that interact with CD47 remain unclear. Furthermore, post-translational modification of CD47 with a glycosaminoglycan chain at Ser⁶⁴ of CD47 is necessary for TSP1 to inhibit CD47 signaling in T cells (Kaur et al., 2011). This modification is prevalent throughout vascular cells, implying that TSP1 signaling through CD47 generally involves interactions with both protein and glycosaminoglycan determinants (Fig. 1).

1.2. CD47 regulation of NO/cGMP signaling

Nitric oxide (NO) plays a central role in cardiovascular physiology to promote arterial dilation and as an anti-thrombotic and anti-inflammatory agent (Ignarro, 2002). Loss of NO synthesis or responsiveness in the cardiovascular system is characteristic of chronic diseases including atherosclerosis, peripheral vascular disease, hypertension, sickle cell disease, and Alzheimer's disease (Chirkov and Horowitz, 2007). Thus, drugs that enhance NO signaling have been developed to improve blood flow, tissue perfusion, and promote angiogenesis in ischemic tissues (Fraisse and Wessel, 2010; Schade et al., 2010; Schmidt et al., 2009). Organonitrates clearly benefit cardiac function (Nossaman et al., 2010), but efforts to use NO-generating drugs or cGMP phosphodiesterase inhibitors to reverse chronic conditions of NO-insufficiency have shown limited benefit. This raises the question of whether signaling downstream of NO is blocked in these chronic vascular diseases.

Endothelium is the primary source of NO in blood vessels. NO produced by endothelial nitric oxide synthase (eNOS, NOS3) (Dudzinski and Michel, 2007) provides a paracrine signal that controls VSMC contractility to regulate blood flow, blood pressure, and tissue perfusion (Stauss et al., 1999). NO activates its primary signaling target soluble guanylyl cyclase (sGC) in VSMC by binding to its prosthetic heme iron, which allosterically accelerates cGMP production several hundred-fold (Arnold et al., 1977; Stone and Marletta, 1994). cGMP activates cGMP dependent kinase-1 (cGK1), which phosphorylates and inhibits myosin light chain kinase and initiates smooth muscle relaxation by allowing dephosphorylation of myosin light chain (MLC-2) (Lincoln et al., 2001). NO/cGMP

signaling stimulates angiogenesis by enhancing endothelial cell chemotaxis and proliferation (Papapetropoulos et al., 1997) and limits platelet activation through cGK1 dependent phosphorylation of targets that limit activation of the platelet integrin $\alpha\text{IIb}\beta 3$ (Butt et al., 1994; Danielewski et al., 2005; Li et al., 2006b).

TSP1 at picomolar concentrations inhibits NO-stimulated activation of sGC in endothelial cells, VSMC, platelets, and T cells (Isenberg et al., 2005b; Isenberg et al., 2008e; Isenberg et al., 2006b; Ramanathan et al., 2011). This activity is lost in CD47 null but not in CD36 null cells and is mimicked by certain ligands of CD47 (Isenberg et al., 2006a). Ligation of CD36 also inhibits sGC activation, but only at higher concentrations of TSP1 and only in cells that express CD47. Tissue cGMP levels are higher in TSP1 and CD47 null mice. Therefore, physiological levels of TSP1 significantly limit NO/cGMP signaling, and CD47 is the primary and necessary receptor to mediate this inhibitory activity. TSP1 also inhibits sGC activation by heme-independent chemical activators (Miller et al., 2010a). TSP1 signaling through CD47 prevents sGC activation by increasing cytoplasmic calcium levels in Jurkat T cells (Ramanathan et al., 2011). CD47 regulates calcium signaling in several other cell types, which regulates eNOS as well as sGC (Bauer et al., 2010; Frazier et al., 2010; Ramanathan et al., 2011).

1.3. Upstream regulation of NO signaling by TSP1

In addition to inhibiting sGC, TSP1 controls NO synthesis (Fig. 2). The activity of eNOS is controlled by mechanical stimuli and by circulating factors such as VEGF and acetylcholine (Dudzinski and Michel, 2007). VEGF signaling through VEGFR2 on endothelial cells activates phosphoinositide 3-kinase, which stimulates Akt activation. Akt phosphorylates eNOS at the activating site Ser¹¹⁷⁷ (Dimmeler et al., 2000). Simultaneously, activated VEGFR2 recruits Src and induces Ca²⁺ signaling, which further activate eNOS (Duval et al., 2007; Gelinas et al., 2002). TSP1 inhibits VEGF signaling via several mechanisms. TSP1 binds to VEGF and prevents VEGFR2 signaling either by competing with extracellular VEGF for binding to cell surface proteoglycans or by promoting VEGF clearance via low density lipoprotein receptor-related protein-1 (LRP1/CD91) (Greenaway et al., 2007; Gupta et al., 1999). Second, TSP1 binding to its receptor CD36 modulates VEGFR2 via a complex involving CD36, the TSP1-binding integrin $\alpha 6\beta 1$, and VEGFR2 (Zhang et al., 2009). Third, CD47 is a proximal binding partner of VEGFR2, and TSP1 binding to CD47 disrupts this complex and inhibits VEGFR2 activation (Kaur et al., 2010; Kaur and Roberts, 2011). Endothelial cells lacking CD47 are resistant to inhibition of VEGFR2 activation by physiological concentrations of TSP1, indicating that this is the dominant pathway under physiological conditions. The other two pathways may become significant under conditions where TSP1 levels are elevated. Downstream activation of Akt phosphorylation was blocked by engaging CD47, consistent with a previous report of increased Akt activation in retinas from TSP1 null mice (Sun et al., 2009). Because CD47 interaction with VEGFR2 globally inhibits VEGF signaling, effects of VEGF signaling on cell motility and survival that are NO-independent can also be inhibited by TSP1 via CD47 (Fig. 2).

Acetylcholine-mediated activation of eNOS in endothelial cells is also inhibited by TSP1 and its CD47-binding domain in a Ca²⁺-dependent manner (Bauer et al., 2010). Exposure of endothelium in WT and TSP1 null arteries but not in CD47 null vessels to soluble TSP1 at physiological concentrations inhibited acetylcholine-stimulated vessel relaxation (Bauer et al., 2010). Conversely, TSP1 potentiated phenylephrine-stimulated vasoconstriction in WT and TSP1 null arteries. In mice lacking TSP1, acetylcholine resulted in a greater decrease in blood pressure compared to WT controls.

1.4. Downstream NO/cGMP target regulation by TSP1

cGK can be activated independent of NO by cell permeable analogs of cGMP, and TSP1 inhibits such activation of cGK in endothelial cells, platelets, and VSMC (Isenberg et al., 2005b; Isenberg et al., 2008e; Isenberg et al., 2006b). This may involve post-translational modification of cGK because inhibition persisted in lysates from TSP1-treated platelets (Isenberg et al., 2008e). Therefore, TSP1 redundantly targets the NO/cGMP signaling cascade and can have direct and indirect effects on vascular cell responses (Fig. 2).

1.5. CD47 cross talk with other TSP1 receptors

CD47 was first isolated based on its co-purification with $\alpha v \beta 3$ integrin, and subsequent studies have revealed specific association of CD47 with additional integrins including $\alpha 2 \beta 1$, $\alpha 4 \beta 1$, $\alpha 6 \beta 1$, and the platelet integrin $\alpha IIb \beta 3$ (Frazier et al., 2010). TSP1 engaging CD47 can induce activation of these integrins. The integrins $\alpha v \beta 3$, $\alpha 6 \beta 1$, and $\alpha 4 \beta 1$ also serve as TSP1 receptors on vascular cells. TSP1 engaging each of these integrins results in proangiogenic signaling in endothelial cells (Calzada et al., 2004). Proliferation and migration of VSMC under normal and hyperglycemic conditions is regulated by TSP1 in a $\alpha v \beta 3$ -dependent manner, and VSMC proliferation was enhanced by ligation of CD47 (Panchatcharam et al., 2010; Isenberg et al., 2005a). Further research is needed to clarify how CD47 modulates TSP1 signaling via these integrins.

CD47 may also participate in vascular signaling via the TSP1 receptor CD36. CD47 is necessary for inhibition of NO signaling by CD36-binding fragments of TSP1 and by the CD36 ligand β -amyloid (Isenberg et al., 2006a; Miller et al., 2010b). This may involve the co-association of CD36 and CD47 with the integrin $\alpha 6 \beta 1$, which was first documented in neural cells (Bamberger et al., 2003).

Evidence in T cells suggests that CD47 also regulates the internalization of TSP1 via calreticulin/LRP1 (Li et al., 2006a). Therefore, CD47 may more broadly regulate TSP1 signaling via all of its receptors by directing its removal from the extracellular space.

1.6. Additional targets of CD47 signaling

cAMP levels in VSMC are in part regulated by cross-talk with cGMP via cGMP-inhibitable cAMP phosphodiesterases, which provides one mechanism for CD47 signaling to control intracellular cAMP levels (Yao et al., 2010). In VSMC and endothelial-free arteries, TSP1 also inhibited adenylate cyclase activation and cAMP accumulation independent of cGMP-mediated cross talk. CD47 ligation also controls cAMP levels by regulation of adenylate cyclase via heterotrimeric G proteins (Frazier et al., 1999).

Based on yeast two-hybrid studies, the short cytoplasmic tail of CD47 interacts with cytoplasmic proteins including PLIC1 and Bcl-2/adenovirus E1B 19-kDa interacting protein (BNIP3) (Frazier et al., 2010). Ligation of CD47 on T cells by TSP1 modulates the interaction between CD47 and BNIP3, which regulates apoptosis by translocating to mitochondria and interacting with Bcl-2 and Bcl-X(L) (Lamy et al., 2007). Reduced T cell apoptosis in TSP1 and CD47 null mice suggested that TSP1 triggers apoptosis via this pathway (Lamy et al., 2007), but TSP1 signaling via CD47 is only implied by the apoptotic activity of extreme concentrations of the modified TSP1 peptide 4N1K (Lamy et al., 2003). CD47 ligation can also stimulate apoptosis by modulation of Fas signaling (Manna et al., 2005), and down-regulation of Drp1 reduced caspase-independent death of leukemic cells induced by soluble TSP1 and a CD47 antibody (Bras et al., 2007). Therefore, TSP1 clearly regulates T cell survival via CD47, but the relative contributions of these three signaling mechanisms remains unclear.

2. Cardiovascular functions of TSP1/CD47 signaling

2.1. Acute effects of TSP1 on tissue perfusion and blood pressure

NO controls regional blood flow by relaxing resistance arteries. Functional MRI of healthy TSP1 null mice showed a greater increase in skeletal muscle blood flow in response to NO within 5 minutes (Isenberg et al., 2007a). Thus, pre-existing levels of TSP1 in the arterial wall or blood constantly limit NO-mediated vasodilation and blood flow. Treatment of isolated pre-contracted VSMCs with TSP1 completely inhibited NO-stimulated relaxation and dephosphorylation of MLC-2. Subsequent studies confirmed that this activity in VSMC is mediated via CD47 (Isenberg et al., 2007b).

Plasma levels of TSP1 in healthy people only partially inhibit eNOS and sGC activation in endothelium. However, plasma TSP1 is known to increase significantly in various disease conditions (Lin et al., 2003; Rico et al., 2010; Smadja et al., 2011). Furthermore, TSP1 levels in the matrix surrounding arterial VSMC increase in atherosclerosis (Riessen et al., 1998; Roth et al., 1998). Thus, direct inhibition of VSMC NO signaling and indirect inhibition via limiting endothelial NO synthesis are CD47-dependent targets that can control vascular tone. Additional TSP1 receptors are present on vascular cells, some of which can oppose these CD47-dependent signals if TSP1 levels are sufficiently elevated (Stenina and Plow, 2008).

Mice lacking or under-expressing eNOS exhibit spontaneous and diet-induced hypertension (Cook et al., 2004). Conversely, inhibiting NO in animals and human subjects raises blood pressure (Broere et al., 1998; Kung et al., 1995). Resting TSP1 null and WT mice had similar mean arterial pressure, but pulse pressure was low in the null mice, suggesting that endogenous TSP1 has a hypertensive activity (Isenberg et al., 2009c). At rest, CD47 null mice are hypotensive with lower mean systolic and diastolic pressures, consistent with enhanced NO signaling and vasodilation. Furthermore, TSP1 and CD47 null mice showed greater decreases in mean arterial blood pressure on treatment with an NO donor or eNOS activator (Bauer et al., 2010; Isenberg et al., 2009c).

Autonomic innervation modulates arterial tone and can be temporarily blocked with hexamethonium. A dose of hexamethonium that induces moderate hypotension in WT mice elicited profound hypotension and death in TSP1 null (Isenberg et al., 2009c) and CD47 null mice (Isenberg, unpublished). These phenotypes of the null mice reveal critical hypertensive roles of TSP1 and CD47 for maintaining central blood pressure under stress. This function is reinforced by evidence that TSP1 has an acute hypertensive activity. Intravenous treatment of WT and TSP1 null mice with TSP1 resulted in increased blood pressure (Bauer et al., 2010). A TSP1 “mimicking” antibody and TSP1 peptides that bind to CD47 similarly increased blood pressure. This hypertensive activity of TSP1 may involve regulation of eNOS activity and antagonism of the hypotensive activity of circulating VEGF (Curwen et al., 2008).

2.2. Effects of TSP1 and CD47 on cardiac dynamics

Several studies have implicated TSP1 in cardiac disease and remodeling, but the TSP1 receptors involved remain unclear (Schellings et al., 2009). The role of NO/cGMP signaling in the heart is also controversial (Garcia-Dorado et al., 2009; Gonzalez et al., 2008). However, cAMP is a potent mediator of both cardiac response and vascular tone (Zaccolo, 2009), and TSP1 and CD47 null cardiac muscle shows increased cAMP levels compared to WT mice (Isenberg et al., 2009c). Cardiac output and ejection fraction increased more in the TSP1 and CD47 null mice in response to systemic vasodilation by an NO donor (Isenberg et al., 2009c). It is unclear, however, whether these enhanced responses are due to loss of TSP1/CD47 signaling in the heart versus in peripheral resistance arteries.

2.3. Platelet hemostasis

NO is a physiological inhibitor of platelet activation (Radomski et al., 1990). In the presence of physiological levels of the NOS substrate arginine or its product NO, platelets from TSP1 null mice were resistant to aggregation induced by thrombin (Isenberg et al., 2008e). Conversely, exogenous TSP1 and CD47 ligands enhanced aggregation under these conditions. Therefore, TSP1 released from platelets provides positive feedback to reinforce platelet aggregation by overcoming the anti-thrombostatic activity of physiological NO concentrations. Thus, the role of elevated TSP1 in wounds may involve two acute activities in addition to long term regulation of angiogenesis and vascular remodeling: 1) TSP1 is a potent vasoconstrictor that initially limits bleeding but can be counterproductive for tissue survival under ischemic stress, and 2) TSP1 is an autocrine factor released by platelets that promotes hemostasis. These insights may also enable us to better understand why TSP1 is down-regulated in many cancers (Isenberg et al., 2009b). Because TSP1 acutely limits tissue perfusion, its expression may be a disadvantage to a growing tumor (Isenberg et al., 2009b). Loss of TSP1 expression may also decrease the thrombogenic potential of the tumor vasculature by maximizing the anti-thrombotic activity of NO produced by the tumor and its stroma.

2.4. Mitochondrial biogenesis

NO/cGMP signaling is a major regulator of mitochondrial biogenesis (Haas et al., 2009; Nisoli and Carruba, 2006). This suggested that mitochondrial homeostasis might be altered in TSP1 and CD47 null mice. Electron microscopy studies revealed increased number and size of mitochondria in skeletal muscle from TSP1 null mice (Frazier et al., 2011). Subsequent work identified similar increases in skeletal muscle mitochondria and in several NO-sensitive regulators of mitochondrial biogenesis in CD47 null mice. Consistent with their increased muscle mitochondrial densities, CD47 null mice exhibit increased exercise endurance and decreased oxygen consumption (Frazier et al., 2011). These studies demonstrate that TSP1/CD47 regulation of physiological NO/cGMP signaling extends beyond the cardiovascular system and has broad metabolic implications.

3. Pathophysiological functions and therapeutic applications

3.1. Functions in injury and stress responses

TSP1/CD47 signaling plays important roles in tumor and wound angiogenesis, fixed ischemic injuries, and ischemia/reperfusion injuries, and readers are referred to recent reviews (Frazier et al., 2010; Isenberg et al., 2008a; Isenberg et al., 2009b; Roberts and Lau, 2011). Elevated plasma TSP1 levels correlate positively with cardiovascular disease (Smadja et al., 2011), suggesting that TSP1 is a biomarker for and potentiator of vascular dysfunction. Recent reports define roles for plasma and tissue TSP1 in promoting pulmonary arterial hypertension (Kaiser et al., 2010; Ochoa et al., 2011; Ochoa et al., 2010). TSP1 null mice exposed to chronic hypoxia exhibited less increase in pulmonary arterial pressure compared to WT mice (Ochoa et al., 2010). Conversely, low plasma TSP1 in human subjects is associated with hypotension (Liu et al., 2008). Although the role of CD47 as a TSP1 receptor in these chronic diseases remains to be established, the TSP1 levels reported should limit NO/cGMP signaling via this receptor.

3.2 Therapeutic opportunities

To date, three approaches have been demonstrated to disrupt the TSP1/CD47 axis in animal disease models. TSP1 can be sequestered or prevented from binding to CD47 using certain TSP1 or CD47 antibodies. The first therapeutic use of a TSP1 antibody was to prevent neointima formation and accelerate re-endothelialization in balloon injured rat carotid arteries (Chen et al., 1999). The TSP1 antibody used, C6.7, was subsequently shown to

inhibit TSP1 interactions with CD47 and increase proliferation of CNS endothelial cells to a similar degree as a CD47 blocking antibody (Myers et al., 2011). The TSP1 antibody A6.1 blocks the ability of TSP1 to inhibit NO-stimulated cGMP accumulation *in vitro* and enhanced survival of ischemic skin flaps in mice and miniature pigs (Isenberg et al., 2007b; Isenberg et al., 2008d). The same TSP1 antibody is radioprotective for human endothelial cells (Maxhimer et al., 2009b), suggesting that it may have therapeutic activity in humans. An anti-mouse CD47 antibody protects animals from ischemic and IR injuries (Isenberg et al., 2008b; Isenberg et al., 2008c; Isenberg et al., 2007b). An anti-rat CD47 antibody similarly protected against IR injury in rats when employed pre- or post- reperfusion (Maxhimer et al., 2009a).

Antisense approaches can be used to temporarily suppress CD47 expression. This approach prevents both TSP1 and SIRP α signaling through CD47 and so may have effects that extend beyond NO/cGMP signaling. Suppression of CD47 expression using a CD47 antisense morpholino protected mice from ischemic and radiation injuries (Isenberg et al., 2008c; Isenberg et al., 2007b; Maxhimer et al., 2009b). The morpholino enhanced survival of ischemic skin flaps in pigs, which better model human ischemic injuries (Isenberg et al., 2008d).

The above results are encouraging for the continued preclinical development of CD47-targeted drugs for treating ischemic injuries, radioprotection, and cancer. These drugs may also be beneficial for treating chronic diseases that are associated with NO insufficiency and elevated TSP1 levels.

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Abbreviations

4N1K	KRFYVVMWKK
BNIP3	Bcl-2/adenovirus E1B 19-kDa interacting protein
cGK	cGMP-dependent protein kinase
IR	ischemia/reperfusion
NO	nitric oxide
eNOS	endothelial nitric oxide synthase
L-NAME	L-N ^G -nitroarginine methyl ester
LRP1	low density lipoprotein receptor-related protein-1
MLC-2	myosin light chain-2
sGC	soluble guanylate cyclase
SIRPα	signal regulatory protein- α
TSP	thrombospondin
VEGFR2	vascular endothelial growth factor receptor-2
VSMC	vascular smooth muscle cell

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Fig. 1. The TSP1 receptor CD47

A model for the interaction of TSP1 with the heparan sulfate modified isoform of CD47 is presented. Modification of CD47 at Ser⁶⁴ is required for inhibitory TSP1 signaling in T cells (Kaur et al., 2011). A long range disulfide bond links the extracellular IgV domain (blue) to the extracellular loop between the transmembrane segments TM4 and TM5 and is required for CD47 signaling (Rebres et al., 2001). BLINK Blast analysis of human CD47 identified multiple presenilins that show significant homology with its transmembrane domain. The lower panel shows alignment of transmembrane domains of xenopus and human presenilin-1 with those of human and mouse CD47. CD47 and presenilin-1 sequences were aligned using the Segment Pair Overlap algorithm of MACAW. Capital letters indicate aligned blocks, and shading indicates conserved residues.

Fig. 2. CD47-mediated regulation of vascular cell signaling by TSP1

Binding of TSP1 to CD47 has a global effect upon the canonical NO pathway. In endothelial cells, TSP1 inhibits VEGFR2 activation through its ability to disrupt the constitutive interactions of CD47 with VEGFR2 (Kaur et al., 2010). This CD47 signal limits Akt-mediated phosphorylation of eNOS at Ser¹¹⁷⁷ and alters endothelial cell calcium transients to prevent calmodulin-mediated activation of eNOS, thereby redundantly suppressing NO production stimulated by VEGF or acetylcholine. CD47 ligation also controls VEGFR2 signaling through the PLCγ pathway and the TSAd adapter to Src that mediate NO-independent endothelial cell growth, migration, and permeability responses. The TSP1/CD47 signal also inhibits heme-dependent and hem-independent activation of the NO cellular target sGC in all vascular cells and T cells (Miller et al., 2010a). TSP1/CD47 signaling independently inhibits the cGMP-stimulated activation of the cGMP target cGK-I. Thus, the TSP1/CD47 axis is a global inhibitor of endogenous and exogenous NO, NO-prodrugs such as nitrite (Isenberg et al., 2009d), and cGMP promoting drugs such as synthetic activators of sGC (YC-1, BAY 41-2272; (Miller et al., 2010a) and cGMP phosphodiesterase inhibitors (e.g. sildenafil).

Fig. 3. Physiological and pathological functions of TSP1/CD47 signaling

At physiological levels of TSP1, signaling through CD47 limits NO/cGMP signaling to support blood pressure, limit tissue perfusion, promote platelet hemostasis, and control mitochondrial biogenesis. Acute or chronic elevation of TSP1 levels in circulation or in tissues contributes to a deficit in NO/cGMP signaling associated with fixed ischemic injuries, ischemia/reperfusion, and several chronic diseases of aging. TSP1/CD47 signaling through cGMP-independent pathways also plays significant roles in some adaptive responses to stress, which involve signaling through additional TSP1 receptors including integrins, CD36, and calreticulin/LRP1.