Published in final edited form as:

J Neurochem. 2009 May ; 109(Suppl 1): 126–132. doi:10.1111/j.1471-4159.2009.05801.x.

CD36: A multi-modal target for acute stroke therapy

Sunghye Cho^{1,2} and Eunhee Kim¹

¹ Burke Medical Research Institute, White Plains, NY 10605

² Department of Neurology/ Neuroscience, Weill Medical College of Cornell University, New York, NY 10021

Abstract

A role for CD36 in the pathogenesis of atherosclerosis, inflammation and lipid metabolism has been well-documented. However, little is known about the role of CD36 in cerebral ischemia. The intent of this review is to develop the concept that CD36, whose functions have been implicated in other pathological events, is a prototypic inflammatory receptor that contributes to the pathogenesis of cerebral ischemia. The importance of CD36 as a treatment target is indicated by the fact that many treatment strategies that are effective in experimental models of stroke exhibit little or no efficacy in clinical trials. The failure of clinical trials may be due to the use of animal models of stroke that do not reflect traditional risk factors for stroke in humans. The discussion will be focused on two risk factors, hyperlipidemia and diabetes, that modulate CD36 responses. Blocking the expression and function of CD36 by pharmacological or genetic means will provide insight not only toward identifying CD36 as a novel molecular target but also for developing effective therapeutic strategies to treat stroke victims. More importantly, coupling clinically relevant conditions with CD36-mediated ischemic injury may provide an appropriate animal model paradigm and develop a scientific understanding that could lead to clinical translational studies involving human subjects.

Introduction

Stroke is a leading cause of death and disability in the U.S. Despite a plethora of studies using animal models of stroke, treatment strategies that target a specific pathway and are effective in these models exhibit little or no efficacy during clinical trials. This may be attributed to the fact that unlike human clinical trials, animals used in preclinical evaluations of stroke may represent a more homogeneous population in terms of gender or age coupled with a relatively consistent degree of injury produced by established animal models. In addition, the pathology of stroke consists of multiple pro-death processes including necrosis, apoptosis, oxidative stress, vascular dysfunction, and pro-inflammatory responses. The interruption of a single pathway in well-controlled model systems thus may not be sufficient to overcome the multifactorial nature of stroke-induced injuries in the human population.

Another underlying issue hindering the development of treatment is the presence of predisposing clinical conditions frequently observed in stroke patients. Multiple cardio- and cerebral-vascular risk factors such as obesity, dyslipidemia, hypertension, insulin/glucose intolerance, and inflammation are associated with increased incidence of stroke either singly

Address correspondence to: Sunghye Cho, Ph.D. Burke/Cornell Medical Research Institute 785 Mamaroneck Avenue White Plains, New York 10605 Email: suc2002@med.cornell.edu Phone: 914–597–2162 Fax: 914–597–2821.

Disclosure : Patent applications have been filed by Cornell Research Foundation, Inc. the technology (SS peptides) described in this article. Sunghye Cho is the co-inventor. Cornell Research Foundation, on behalf of Cornell University, has licensed the technology for further research and development to a commercial enterprise in which CRF has financial interests.

or in association with the clustering of other risk factors. More importantly, these additional clinical conditions may impact disease progression and outcomes of stroke-induced injury. Whereas less favorable outcomes are predicted in patients with predisposing clinical conditions, systematic inclusion of these risk factors in animal models may provide the next level of understanding of stroke pathology in clinical settings.

CD36, an 88 kDa glycoprotein, was originally described as platelet receptor glycoprotein and belongs to the class B scavenger receptor family. Existing knowledge of CD36 biology in other systems led us to formulate hypothesis that CD36 contributes to acute stroke pathophysiology. The review will discuss the evidence supporting the consideration of multimodal approaches and an inclusion of predisposing clinical conditions in an experimental model of stroke for an effective translation, particularly how CD36 fulfills both recommendations.

Multimodal approaches

To overcome the heterogeneous pathophysiology associated with stroke, multimodal approaches may be an appropriate strategy for treatment. Studies have shown that a combination of neuroprotective agents commonly used for stroke prevention effectively reduced post-stroke injury (Chen *et al.* 2004, Ding *et al.* 2005). Other promising multi-targeted approaches involve the transcriptional enhancement of genes that are responsible for pro-survival functions. Promoting histone acetylation using histone deacetylase inhibitors (Langley *et al.* 2005) or inhibitors of prolyl hydroxylase domain-containing proteins (Siddiq *et al.* 2005) resulting in protection from oxidative death and ischemic injury. This type of transcriptional approach has been suggested as a potential strategy for neuroprotection (Ratan *et al.* 2004).

Comparable to the above mentioned approaches, a strategy may be aimed at a single target that is implicated in multiple pathogenic downstream pathways. CD36 is a multifunctional receptor that is involved in numerous cellular processes. Many of these CD36-regulated processes are closely associated with pathological features frequently observed in neurodegenerative/neurological diseases.

Role of CD36 in acute stroke pathology

CD36 is a multifunctional receptor that is involved in inflammation, angiogenesis, lipid metabolism and atherosclerosis. It is expressed in various types of cells and tissues including microglia and astrocytes in the brain, monocytes/macrophages, platelets, microvascular endothelium, cardiac and skeletal muscle, adipocytes, dendritic cells, and epithelia of the retina, breast, and kidney. CD36 exhibits high affinity towards a wide variety of structurally distinct ligands, including oxidized or modified low density lipoprotein (oxLDL, mLDL), long-chain fatty acid (LCFA), thrombospondin (TSP) –1 and –2, fibrillar β -amyloid (fA β), *Plasmodium falciparum*-parasitized erythrocytes, and membranes of cells undergoing apoptosis (for detailed review see (Febbraio *et al.* 2001, Febbraio & Silverstein 2007).

In spite of its low expression in the normal brain, CD36 expression is up-regulated following cerebral ischemia. Detailed immunohistochemical studies in the brain have shown that CD36 expression occurs mainly on CD11b+ microglia or infiltrated macrophages within the infarct territory throughout infarct development (Cho *et al.* 2005). CD36 staining is undetectable in neurons, whereas astrocytic expression is limited to the peri-infarct area at the time the glial scar forms. The differential expression of CD36, either temporally or spatially, in the specific subsets of cells following ischemia suggests that CD36 may play diverse roles in stroke pathology. As a result of its expression in multiple cell types and a host of ligands produced in the ischemic brain, the activation of CD36 may have a

significant impact on the progression and outcome of stroke (Figure 1). Targeting CD36 may permit concurrent mitigation of numerous pathogenic processes to achieve global neuroprotection.

a. Angiogenesis

Angiogenesis plays a critical role in stroke outcome and recovery. Promotion of ischemia-induced angiogenesis, especially within the ischemic boundary, has been suggested as a therapeutic strategy to improve stroke outcome (Slevin *et al.* 2006).

Angiogenesis is tightly regulated by balancing the expression of factors that promotes as well as inhibits the formation of blood vessels. TSP-1 and 2 are extracellular matrix proteins and ligands for CD36. Binding of TSPs to CD36 mediates a signaling cascade that leads to endothelial cell apoptosis (Jimenez *et al.* 2000). The relevance of CD36 function in stroke pathology is its anti-angiogenic nature, which offsets compensatory angiogenesis-promoting cascades in response to tissue ischemia. With increased expression of CD36 and TSPs following ischemia (Lin *et al.* 2003, Hayashi *et al.* 2003, Cho *et al.* 2005), angiostatic signaling via excessive TSPs-CD36 interaction may contribute to the stroke pathology. A previous study showed that CD36 KO mice exhibited smaller infarct size and less behavior deficit (Cho *et al.* 2005). Whether the better ischemic outcome is attributed to the enhanced angiogenesis in the peri-infarct area remained to be investigated. By virtue of improving cerebral blood flow in the post-ischemic brain, blocking TSPs-CD36 interaction would be a potential strategy for stroke therapy.

b. Innate immunity

Recently, much attention has been given to the understanding of immune responses following cerebral ischemia. Isolated by the blood brain barrier, the brain has historically been considered an immune privileged organ. However, ischemia-induced blood brain barrier breakdown and subsequent exposure of CNS antigens to the infiltrating peripheral immune cells confirm the presence of all the components needed to trigger immune responses in the post-ischemic brain. The outcomes of the elicited immune responses are uncertain and may result in either protection (Ziv *et al.* 2007) or destruction (Gee *et al.* 2007). As the most primordial function in the organism, innate immunity serves to safeguard host tissue through the recognition of modified or oxidized phosphatidylcholine/serine motifs on the surface of apoptotic cells followed by subsequent removal of apoptotic and senescent cells (Hazen 2008). Triggered by tissue damage or infection by pathogenic or non-pathogenic microbes, the innate immune system appears to mediate ensuing inflammatory responses via a family of pattern recognition receptors. The receptors include toll-like receptors (TLRs) and CD36 which may recognize endogenous danger signals from damaged tissues (Matzinger 2002) or a host of structural motifs termed “pathogen-associated molecular pattern” for phagocytosis (Savill 1997).

The pathogenic roles of CD36 and TLR2 in cerebral ischemia have been independently reported. CD36 expression increases in the post-ischemic brain, and the targeted disruption of *cd36* in mice reduces ischemic injury (Cho *et al.* 2005). Similarly, TLR2 expression is increased during ischemia and deletion of *tlr2* results in reduced ischemic injury (Ziegler *et al.* 2007). A study showed that CD36 is a sensor of diacylated bacterial lipoprotein and a co-receptor for TLR2/6 (Hoebe *et al.* 2005). The closely shared function and physical association between CD36 and TLRs suggest that CD36 is involved in innate immune responses following cerebral ischemia. Targeting CD36 in an attempt to attenuate innate immune responses under pathological conditions may be a potential strategy for stroke therapy. Of particular note is that clearance of apoptotic cell bodies by phagocytosis, an essential function of scavenger receptors, may be an important process for tissue remodeling

and wound healing after injury. Therefore, despite the potential damaging role of CD36 in acute ischemia, a role for CD36 in stroke recovery and remodeling process require further investigation.

c. Inflammation

Inflammation is a rapid and coordinated process in response to microbial infection. However, sterile inflammation, an inflammatory response in the absence of infection, also occurs in the setting of cerebral ischemia and trauma. Primary input to trigger sterile inflammatory responses is linked to the innate immune system through pattern recognition receptors (Mollen *et al.* 2006).

The pro-inflammatory nature of CD36 has been implicated in atherosclerosis (Febbraio *et al.* 2000), Alzheimer's disease (El Khoury *et al.* 2003), and Parkinson's disease (Su *et al.* 2007). In cerebral ischemia, CD36 mediates free radical generation and contributes to tissue injury in the post-ischemic brain (Cho *et al.* 2005). Using the cell permeable anti-oxidant peptide SS31 that down-regulates CD36 pathways, our laboratory demonstrated reduced injury size and GSH depletion in wild-type, but not in CD36 null mice after ischemia (Cho *et al.* 2007). A subsequent study reported a shift toward less pro-inflammatory state in the post-ischemic brain in the absence of CD36 and suggested that CD36 is a critical mediator to elicit pro-inflammatory responses following ischemia (Kim *et al.* 2008). Complementary to these studies, Kunz and colleagues reported reduced NF- κ B activation in CD36 KO mice in the post ischemic brain (Kunz *et al.* 2008). The pro-inflammatory responses associated with generation of free radicals, cytokines, chemokines and foam cells through interaction between CD36/CD36 complex and ligands (oxLDL, fA β) may be an integral part of many pathological processes and strategies may be aimed at attenuating injury-induced inflammation by targeting CD36.

d. Lipid metabolism

Yang and colleagues suggest a deleterious effect of long chain fatty acids on ischemic/reperfusion injury (Yang *et al.* 2007). In the cerebrospinal fluid from stroke patients, there is a significant increase in free fatty acids and increased lipid levels were shown to associate with poor ischemic outcomes (Pilitsis *et al.* 2003).

CD36 facilitates long chain fatty acids transfer across the plasma membrane (Abumrad *et al.* 1993). Because CD36 was also found on mitochondrial, an essential role of CD36 in fatty acid oxidation has been suggested (Bezaire *et al.* 2006). A recent study showed that enhanced fatty acid oxidation causes sub-cellular shuffling of CD36 to mitochondria (Benton *et al.* 2008). As reported in heart, hypoxia also induces the translocation of CD36 from the intracellular pool to the plasma membrane resulting in intracellular lipid accumulation (Chabowski *et al.* 2006). Intramyocardial lipid overload contributes to contractile dysfunction and ischemia-reperfusion injury (Korge *et al.* 2003). Although evidence of intracellular lipotoxicity in post-ischemic brain is currently lacking, it would be important to address whether elevated free fatty acids and intracellular lipid accumulation following cerebral ischemia is associated with CD36 and its subcellular localization. Whether this event negatively influences ischemic-reperfusion injury in the brain remains to be determined.

Clinical conditions that modulate CD36 expression and functions

The lack of translational efficacy of treatments used in rodents has prompted a reevaluation of experimental stroke models. In-depth analyses of critical issues including statistical power and reproducibility, statistical analysis, randomization, quality-control mechanisms, and bias on negative results have been recently addressed (Dirnagl 2006). Another relevant

recommendation is inclusion of prevailing risk factors in the experimental models of stroke. Among predisposing clinical conditions associated with stroke patients, we will limit our discussion to hyperlipidemic and diabetic conditions because expression and function of CD36 changes in these conditions. Understanding how these conditions modulate ischemic outcome in relation to CD36 in a preclinical setting may provide a platform to develop strategies for effective translation.

a. Hyperlipidemia

Ligand-receptor interactions markedly induce CD36 gene and protein expression via peroxisome proliferators activated receptor (PPAR)- γ (Nagy *et al.* 1998), and promotes uptake of oxLDLs that leads to foam cell formation and development of atherosclerosis. A proatherogenic role for CD36 is further supported by the reduced lesion development in its absence (Febbraio *et al.* 2000, Guy *et al.* 2007, Moore *et al.* 2005). Generating excess lipid-based CD36 ligands in hyperlipidemic conditions may contribute to enhanced inflammation and may be linked to the pathology of cerebral ischemia. Our laboratory showed that hyperlipidemic mice showed larger infarct size, a disproportionally enlarged swelling, increased lipid content, formation of numerous foam cells (Figure 2), and heightened inflammation in the brain compared to the normolipidemic mice. Another intriguing finding from the study was that these foam cells were primarily localized in the peri-infarct area where post-ischemic inflammation predominates. Deletion of *cd36* gene in these hyperlipidemic mice resulted in reduced infarct size and swelling, less foam cell formation, and reduced pro-inflammatory responses (Kim *et al.* 2008). The reversal of the pro-inflammatory phenotype in these mice suggests that CD36-mediated pathways are partly accountable for the exacerbation of ischemic injury in hyperlipidemic conditions.

Another possible link between CD36 and lipids may be inferred from intervention studies with statins that show protection against cerebral ischemia (Sugiura *et al.* 2007). Although the protective effects are attributed to the pleiotropic actions of statins, the drugs attenuate CD36 expression/oxLDL uptake in inflammatory cells, increase cholesterol efflux, and decrease cholesterol ester accumulation (Fuhrman *et al.* 2002, Llaverias *et al.* 2004). Whether blocking the CD36-mediated lipid burden by statins or other pharmacological interventions is protective in hyperlipidemic conditions remains to be determined. However, the approach will confirm whether CD36-mediated excess lipid uptake contributes to the exacerbation of stroke-induced injury or not in hyperlipidemic conditions.

b. Diabetes

Studies show that 70% of new stroke victims were previously diagnosed with diabetes, occult diabetes, or were pre-diabetic (Ruiz-Velasco *et al.* 2004). Among non-diabetic patients with a history of a recent transient ischemic attack or ischemic stroke, impaired insulin sensitivity or insulin resistance was a prevalent condition (Argmann *et al.* 2005). There is a striking association between diabetes and the induction of CD36 expression. For instance, CD36 expression occurs in tissue or cells where diabetic complications prevail, such as the heart, microvascular endothelial cells, retinal pigment epithelium, and proximal renal tubular epithelial cells.

CD36 expression is increased in monocytes from type II diabetic patients (Kernan *et al.* 2003) and in the cardiac tissue of diabetic mice (Susztak *et al.* 2005). These studies are consistent with a report showing that impaired insulin signaling increases CD36 in macrophages isolated from ob/ob diabetic mice (Sampson *et al.* 2003). In addition to oxidative modification, LDLs are modified by glycation in the presence of high glucose. The resulting glycated-oxidized (glycoxidized) LDLs stimulate macrophage proliferation in a CD36-dependent manner and increase CD36 gene expression and cholesterol accumulation

in THP-1 macrophages (Lam *et al.* 2004). By inference, the diabetic condition and/or hyperglycemia would be expected to exacerbate ischemic inflammation and injury via the augmented burden of CD36 ligands and function (Figure 3). Understanding the mechanism by which CD36 pathways are modulated in the experimental diabetic conditions will strengthen the link between the induction of CD36 and hyperglycemia and support the rationale for inclusion of the experimental diabetic conditions in the preclinical setting.

Targeting CD36 for pharmacological intervention

Hexarelin, a member of the hexapeptide growth hormone-releasing peptides (GHRPs), binds to CD36 receptors (Demers *et al.* 2004). Treatment of mice with hexarelin or a structurally related analogue, EP80317, results in a marked decrease in atherosclerotic lesions (Marleau *et al.* 2005). Hexarelin exerts its protective effect by blocking CD36-mediated uptake of oxLDLs through binding site competition. Other promising antagonists against scavenger receptors are synthetically engineered nanoblockers that block the major scavenger receptors from oxLDLs uptake and thus prevent formation of foam cells (Chnari *et al.* 2006).

Besides targeting oxLDL binding sites, other approaches have been used to modulate CD36 expression or its downstream effects. Statins or antioxidants such as α -tocopherol, can reduce expression of CD36 and the uptake of oxLDLs into macrophages (Ricciarelli *et al.* 2000, Fuhrman *et al.* 2002). Another new class of antioxidants, SS peptides, significantly reduces reperfusion arrhythmia, myocardial stunning, and infarct size *in vivo*. The potency of the SS peptide antioxidants is attributed to their ability to concentrate within the inner mitochondrial membrane and inhibit the mitochondrial permeability transition, mitochondrial swelling, cytochrome *c* release, and apoptosis induced by lipid peroxides (Szeto 2006). Our laboratory has recently investigated the efficacy of SS31 on stroke. Treatment of mice with the SS31 peptide attenuated ischemia-induced GSH depletion in the cortex and reduced infarct size. By contrast, the protective effect of SS31 was abrogated in CD36 knock-out mice, indicating that its protective effect occurs at the level of CD36 via down regulating CD36-mediated pathways (Cho *et al.* 2007). The strong link between manifestations of oxidative stress and CD36 expression suggests that targeting CD36 may hold promise for a useful strategy treating acute ischemic stroke.

Summary

This review has provided evidence that CD36, functioning in the context of previously recognized pathological events, is linked to the pathology of cerebral ischemia including angiostasis, innate immunity and inflammation and lipid metabolism. In addition to these key functions, CD36 expression is closely associated with certain risk factors such as hyperlipidemia and diabetes. The concept that the predisposing clinical conditions modulate the extent of stroke-induced injury suggests an inclusion of experimental hyperlipidemic and diabetic conditions in animal models of stroke. Because CD36 is an inflammatory associated receptor that is at the crossroads of cardio- and cerebro-vascular diseases, intervening CD36-mediated inflammation and blocking its functions will provide insight whether the receptor serves as a novel molecular target to treat acute stroke.

Acknowledgments

This work was supported by NIH grant NHLBI RO1 HL082511 and The Burke Foundation.

References

- Abumrad NA, el-Maghrabi MR, Amri EZ, Lopez E, Grimaldi PA. Cloning of a rat adipocyte membrane protein implicated in binding or transport of long-chain fatty acids that is induced during

- preadipocyte differentiation. Homology with human CD36. *J Biol Chem.* 1993; 268:17665–17668. [PubMed: 7688729]
- Argmann CA, Edwards JY, Sawyez CG, O'Neil CH, Hegele RA, Pickering JG, Huff MW. Regulation of macrophage cholesterol efflux through hydroxymethylglutaryl-CoA reductase inhibition: a role for RhoA in ABCA1-mediated cholesterol efflux. *J Biol Chem.* 2005; 280:22212–22221. [PubMed: 15817453]
- Benton CR, Holloway GP, Campbell SE, Yoshida Y, Tandon NN, Glatz JF, Luiken JJ, Spriet LL, Bonen A. Rosiglitazone increases fatty acid oxidation and fatty acid translocase (FAT/CD36) but not carnitine palmitoyltransferase I in rat muscle mitochondria. *J Physiol.* 2008; 586:1755–1766. [PubMed: 18238811]
- Bezair V, Bruce CR, Heigenhauser GJ, Tandon NN, Glatz JF, Luiken JJ, Bonen A, Spriet LL. Identification of fatty acid translocase on human skeletal muscle mitochondrial membranes: essential role in fatty acid oxidation. *Am J Physiol Endocrinol Metab.* 2006; 290:E509–515. [PubMed: 16219667]
- Chabowski A, Gorski J, Calles-Escandon J, Tandon NN, Bonen A. Hypoxia-induced fatty acid transporter translocation increases fatty acid transport and contributes to lipid accumulation in the heart. *FEBS Lett.* 2006; 580:3617–3623. [PubMed: 16753149]
- Chen J, Li Y, Zhang R, Katakowski M, Gautam SC, Xu Y, Lu M, Zhang Z, Chopp M. Combination therapy of stroke in rats with a nitric oxide donor and human bone marrow stromal cells enhances angiogenesis and neurogenesis. *Brain Res.* 2004; 1005:21–28. [PubMed: 15044060]
- Chnari E, Nikitczuk JS, Wang J, Uhrich KE, Moghe PV. Engineered polymeric nanoparticles for receptor-targeted blockage of oxidized low density lipoprotein uptake and atherogenesis in macrophages. *Biomacromolecules.* 2006; 7:1796–1805. [PubMed: 16768400]
- Cho S, Park EM, Febbraio M, Anrather J, Park L, Racchumi G, Silverstein RL, Iadecola C. The class B scavenger receptor CD36 mediates free radical production and tissue injury in cerebral ischemia. *J Neurosci.* 2005; 25:2504–2512. [PubMed: 15758158]
- Cho S, Szeto HH, Kim E, Kim H, Tolhurst AT, Pinto JT. A Novel Cell-permeable Antioxidant Peptide, SS31, Attenuates Ischemic Brain Injury by Down-regulating CD36. *J Biol Chem.* 2007; 282:4634–4642. [PubMed: 17178711]
- Demers A, McNicoll N, Febbraio M, Servant M, Marleau S, Silverstein R, Ong H. Identification of the growth hormone-releasing peptide binding site in CD36: a photoaffinity cross-linking study. *Biochem J.* 2004; 382:417–424. [PubMed: 15176951]
- Ding G, Jiang Q, Zhang L, Zhang ZG, Li L, Knight RA, Ewing JR, Wang Y, Chopp M. Analysis of combined treatment of embolic stroke in rat with r-tPA and a GPIIb/IIIa inhibitor. *J Cereb Blood Flow Metab.* 2005; 25:87–97. [PubMed: 15678115]
- Dirnagl U. Bench to bedside: the quest for quality in experimental stroke research. *J Cereb Blood Flow Metab.* 2006; 26:1465–1478. [PubMed: 16525413]
- El Khoury JB, Moore KJ, Means TK, Leung J, Terada K, Toft M, Freeman MW, Luster AD. CD36 mediates the innate host response to beta-amyloid. *J Exp Med.* 2003; 197:1657–1666. [PubMed: 12796468]
- Febbraio M, Hajjar DP, Silverstein RL. CD36: a class B scavenger receptor involved in angiogenesis, atherosclerosis, inflammation, and lipid metabolism. *J Clin Invest.* 2001; 108:785–791. [PubMed: 11560944]
- Febbraio M, Podrez EA, Smith JD, Hajjar DP, Hazen SL, Hoff HF, Sharma K, Silverstein RL. Targeted disruption of the class B scavenger receptor CD36 protects against atherosclerotic lesion development in mice. *J Clin Invest.* 2000; 105:1049–1056. [PubMed: 10772649]
- Febbraio M, Silverstein RL. CD36: implications in cardiovascular disease. *Int J Biochem Cell Biol.* 2007; 39:2012–2030. [PubMed: 17466567]
- Fuhrman B, Koren L, Volkova N, Keidar S, Hayek T, Aviram M. Atorvastatin therapy in hypercholesterolemic patients suppresses cellular uptake of oxidized-LDL by differentiating monocytes. *Atherosclerosis.* 2002; 164:179–185. [PubMed: 12119208]
- Gee JM, Kalil A, Shea C, Becker KJ. Lymphocytes: potential mediators of postischemic injury and neuroprotection. *Stroke.* 2007; 38:783–788. [PubMed: 17261738]

- Guy E, Kuchibhotla S, Silverstein R, Febbraio M. Continued inhibition of atherosclerotic lesion development in long term Western diet fed CD36⁰/apoE⁰ mice. *Atherosclerosis*. 2007; 192:123–130. [PubMed: 16919281]
- Hayashi T, Noshita N, Sugawara T, Chan PH. Temporal profile of angiogenesis and expression of related genes in the brain after ischemia. *J Cereb Blood Flow Metab*. 2003; 23:166–180. [PubMed: 12571448]
- Hazen SL. Oxidized phospholipids as endogenous pattern recognition ligands in innate immunity. *J Biol Chem*. 2008
- Hoebe K, Georgel P, Rutschmann S, et al. CD36 is a sensor of diacylglycerides. *Nature*. 2005; 433:523–527. [PubMed: 15690042]
- Jimenez B, Volpert OV, Crawford SE, Febbraio M, Silverstein RL, Bouck N. Signals leading to apoptosis-dependent inhibition of neovascularization by thrombospondin-1. *Nat Med*. 2000; 6:41–48. [PubMed: 10613822]
- Kernan WN, Inzucchi SE, Viscoli CM, Brass LM, Bravata DM, Shulman GI, McVeety JC, Horwitz RI. Impaired insulin sensitivity among nondiabetic patients with a recent TIA or ischemic stroke. *Neurology*. 2003; 60:1447–1451. [PubMed: 12743229]
- Kim E, Tolhurst AT, Qin LY, Chen XY, Febbraio M, Cho S. CD36/fatty acid translocase, an inflammatory mediator, is involved in hyperlipidemia-induced exacerbation in ischemic brain injury. *J Neurosci*. 2008; 28:4661–4670. [PubMed: 18448643]
- Korge P, Honda HM, Weiss JN. Effects of fatty acids in isolated mitochondria: implications for ischemic injury and cardioprotection. *Am J Physiol Heart Circ Physiol*. 2003; 285:H259–269. [PubMed: 12793979]
- Kunz A, Abe T, Hochrainer K, Shimamura M, Anrather J, Racchumi G, Zhou P, Iadecola C. Nuclear factor-kappaB activation and postischemic inflammation are suppressed in CD36-null mice after middle cerebral artery occlusion. *J Neurosci*. 2008; 28:1649–1658. [PubMed: 18272685]
- Lam MC, Tan KC, Lam KS. Glycooxidized low-density lipoprotein regulates the expression of scavenger receptors in THP-1 macrophages. *Atherosclerosis*. 2004; 177:313–320. [PubMed: 15530905]
- Langley B, Gensert JM, Beal MF, Ratan RR. Remodeling chromatin and stress resistance in the central nervous system: histone deacetylase inhibitors as novel and broadly effective neuroprotective agents. *Curr Drug Targets CNS Neurol Disord*. 2005; 4:41–50. [PubMed: 15723612]
- Lin TN, Kim GM, Chen JJ, Cheung WM, He YY, Hsu CY. Differential regulation of thrombospondin-1 and thrombospondin-2 after focal cerebral ischemia/reperfusion. *Stroke*. 2003; 34:177–186. [PubMed: 12511771]
- Llaverias G, Lacasa D, Vinals M, Vazquez-Carrera M, Sanchez RM, Laguna JC, Alegret M. Reduction of intracellular cholesterol accumulation in THP-1 macrophages by a combination of rosiglitazone and atorvastatin. *Biochem Pharmacol*. 2004; 68:155–163. [PubMed: 15183127]
- Marleau S, Harb D, Bujold K, et al. EP 80317, a ligand of the CD36 scavenger receptor, protects apolipoprotein E-deficient mice from developing atherosclerotic lesions. *Faseb J*. 2005; 19:1869–1871. [PubMed: 16123174]
- Matzinger P. The danger model: a renewed sense of self. *Science*. 2002; 296:301–305. [PubMed: 11951032]
- Mollen KP, Anand RJ, Tsung A, Prince JM, R.M. I, T.R. B. Emerging paradigm: Toll-like receptor 4-sentinel for the detection of tissue damage. *Shock*. 2006; 26:430–437. [PubMed: 17047512]
- Moore KJ, Kunjathoor VV, Koehn SL, Manning JJ, Tseng AA, Silver JM, McKee M, Freeman MW. Loss of receptor-mediated lipid uptake via scavenger receptor A or CD36 pathways does not ameliorate atherosclerosis in hyperlipidemic mice. *J Clin Invest*. 2005; 115:2192–2201. [PubMed: 16075060]
- Nagy L, Tontonoz P, Alvarez JG, Chen H, Evans RM. Oxidized LDL regulates macrophage gene expression through ligand activation of PPARgamma. *Cell*. 1998; 93:229–240. [PubMed: 9568715]
- Pilitsis JG, Coplin WM, O'Regan MH, Wellwood JM, Diaz FG, Fairfax MR, Michael DB, Phillis JW. Measurement of free fatty acids in cerebrospinal fluid from patients with hemorrhagic and ischemic stroke. *Brain Res*. 2003; 985:198–201. [PubMed: 12967724]

- Ratan RR, Siddiq A, Aminova L, Lange PS, Langley B, Ayoub I, Gensert J, Chavez J. Translation of ischemic preconditioning to the patient: prolyl hydroxylase inhibition and hypoxia inducible factor-1 as novel targets for stroke therapy. *Stroke*. 2004; 35:2687–2689. [PubMed: 15472113]
- Ricciarelli R, Zingg JM, Azzi A. Vitamin E reduces the uptake of oxidized LDL by inhibiting CD36 scavenger receptor expression in cultured aortic smooth muscle cells. *Circulation*. 2000; 102:82–87. [PubMed: 10880419]
- Ruiz-Velasco N, Dominguez A, Vega MA. Statins upregulate CD36 expression in human monocytes, an effect strengthened when combined with PPAR-gamma ligands Putative contribution of Rho GTPases in statin-induced CD36 expression. *Biochem Pharmacol*. 2004; 67:303–313. [PubMed: 14698043]
- Sampson MJ, Davies IR, Braschi S, Ivory K, Hughes DA. Increased expression of a scavenger receptor (CD36) in monocytes from subjects with Type 2 diabetes. *Atherosclerosis*. 2003; 167:129–134. [PubMed: 12618277]
- Savill J. Recognition and phagocytosis of cells undergoing apoptosis. *Br Med Bull*. 1997; 53:491–508. [PubMed: 9374033]
- Siddiq A, Ayoub IA, Chavez JC, et al. Hypoxia-inducible factor prolyl 4-hydroxylase inhibition. A target for neuroprotection in the central nervous system. *J Biol Chem*. 2005; 280:41732–41743. [PubMed: 16227210]
- Slevin M, Kumar P, Gaffney J, Kumar S, Krupinski J. Can angiogenesis be exploited to improve stroke outcome? Mechanisms and therapeutic potential. *Clin Sci (Lond)*. 2006; 111:171–183. [PubMed: 16901264]
- Su X, Maguire-Zeiss KA, Giuliano R, Prifti L, Venkatesh K, Federoff HJ. Synuclein activates microglia in a model of Parkinson's disease. *Neurobiol Aging*. 2007
- Sugiura S, Yagita Y, Sasaki T, Todo K, Terasaki Y, Ohyama N, Hori M, Kitagawa K. Postischemic administration of HMG CoA reductase inhibitor inhibits infarct expansion after transient middle cerebral artery occlusion. *Brain Res*. 2007; 1181:125–129. [PubMed: 17920046]
- Susztak K, Ciccone E, McCue P, Sharma K, Bottinger EP. Multiple metabolic hits converge on CD36 as novel mediator of tubular epithelial apoptosis in diabetic nephropathy. *PLoS Med*. 2005; 2:e45. [PubMed: 15737001]
- Szeto HH. Cell-permeable, mitochondrial-targeted, peptide antioxidants. *AAPS J*. 2006; 8:E277–283. [PubMed: 16796378]
- Yang DY, Pan HC, Yen YJ, et al. Detrimental effects of post-treatment with fatty acids on brain injury in ischemic rats. *Neurotoxicology*. 2007; 28:1220–1229. [PubMed: 17854901]
- Ziegler G, Harhausen D, Schepers C, et al. TLR2 has a detrimental role in mouse transient focal cerebral ischemia. *Biochem Biophys Res Commun*. 2007; 359:574–579. [PubMed: 17548055]
- Ziv Y, Finkelstein A, Geffen Y, et al. A novel immune-based therapy for stroke induces neuroprotection and supports neurogenesis. *Stroke*. 2007; 38:774–782. [PubMed: 17261737]

Figure 1. A proposed multimodal strategy for stroke using CD36 as a target

A, The binding of thrombospondins (TSPs) elicits an anti-angiogenic signaling cascade that causes apoptosis of endothelial cells. By recognizing pathogen-associated molecular patterns of dying cells, CD36 is involved in innate immunity. In response to fibrillar A β (fA β), CD36 mediates an innate host response. Foam cell formation by uptake of oxLDLs contributes to atherosclerotic lesion development. CD36 translocates long chain fatty acids (LCFA) across the plasma and mitochondrial membrane and involved in lipid metabolism. Ligands/receptor interactions markedly induce CD36 gene and protein expression in a feed forward manner and results in pathological consequences. **B**, Blocking CD36 by genetic or pharmacological means results in interruption of the feed forward loop, down-regulates CD36 pathways, and leads to global protection against pathological conditions such as stroke. L, ligands; Inh, Inhibitors

Figure 2. Hyperlipidemia-promoted foam cell formation

Oil-red O staining in the ipsilateral side of normolipidemic (NL) and hyperlipidemic (HL) brain 3 days following ischemia. No foam cells were observed in the normolipidemic brain. On the contrary, there are numerous oil-red O stained foam cells in the penumbra from the hyperlipidemic brain. The lower panels show the higher magnification of the boxed areas. Scale bar, 500 μ m

Figure 3. CD36 exacerbates ischemic injury in predisposing clinical conditions

Upon ischemia-reperfusion in normal condition, CD36 ligands such as free fatty acids (FFAs) and oxLDLs are generated. Interactions between CD36 and the liberated ligands generate reactive oxygen/nitrogen species and pro-inflammatory mediators, and contribute to ischemia-induced injury. Excessive ligands are generated in hyperlipidemic (FFAs, oxLDLs) and diabetic conditions (advanced glycated endproducts (AGEs), glycated-oxLDL(Glyc-oxLDL)). Enhanced receptor/ligand interactions further increase CD36 expression and the feed forward loop exacerbates ischemia-induced inflammation and injury. FFA, free fatty acid; AGE, advanced glycated end products; Glyc-oxLDL, glycated-oxidized LDL