Protective Role of Kallistatin in Vascular and Organ Injury

Julie Chao, Grant Bledsoe, Lee Chao

Kallistatin is an endogenous protein that exerts pleiotropic effects, including vasodilation and inhibition of angiogenesis, inflammation, oxidative stress, apoptosis, fibrosis, and tumor progression. Through its 2 functional domains—an active site and a heparin-binding site—kallistatin regulates differential signaling pathways and a wide spectrum of biological functions. Kallistatin’s active site is key for inhibiting tissue kallikrein activity and stimulating the expression of endothelial nitric oxide (NO) synthase (eNOS), sirtuin 1 (SIRT1), and suppressor of cytokine signaling 3 (SOCS3). Kallistatin via its heparin-binding site blocks signaling pathways mediated by growth factors and cytokines, such as vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), high-mobility group box-1 (HMGB1), Wnt, transforming growth factor-β (TGF-β), and epidermal growth factor. Kallistatin gene or protein delivery protects against the pathogenesis of hypertension, heart and kidney damage, arthritis, sepsis, influenza virus infection, tumor growth, and metastasis in animal models. Conversely, depletion of endogenous kallistatin by neutralizing antibody injection exacerbates cardiovascular and renal injury in hypertensive rats. Kallistatin levels are markedly reduced in rodents with hypertension, sepsis, streptozotocin-induced diabetes mellitus, and cardiac and renal injury. Kallistatin levels are also diminished in patients with liver disease, septic syndrome, diabetic retinopathy, severe pneumonia, inflammatory bowel disease, obesity, and prostate, and colon cancer. Therefore, circulating kallistatin levels may serve as a new biomarker for human diseases. This review summarizes kallistatin’s protective roles and mechanisms in vascular and organ injury and highlights the therapeutic potential of kallistatin for multiple disease states.

Kallistatin Regulates Biological Functions via Its Structural Elements

Kallistatin was discovered in human plasma as a tissue kallikrein–binding protein. Sequence analysis indicated that tissue kallikrein–binding protein is a novel serine proteinase inhibitor (serpin) and was later designated kallistatin because of its ability to inhibit tissue kallikrein activity. Tissue kallikrein is a serine proteinase that cleaves low–molecular weight kininogen substrate to release vasoactive kinin peptides. The tissue kallikrein–kinin system is involved in beneficial effects against hypertension and cardiac, cerebral, and renal injury. Kallistatin is mainly expressed in the liver, but is also widely distributed in tissues relevant to cardiovascular function, including the heart, kidney, and blood vessel. The human kallistatin gene (SERPINA4) is located on chromosome 14q31-32.1 within a serpin gene cluster, and human kallistatin cDNA shares 44% to 46% homology with other serpins, such as α1-antitrypsin, α1-antichymotrypsin, and protein C inhibitor. Kallistatin contains 2 important structural elements: an active site and a heparin-binding domain. Through these 2 distinct structural elements, kallistatin regulates the expression of multiple genes and controls the activation of several signaling pathways. Kallistatin’s active site is crucial for (1) inhibiting tissue kallikrein activity and tissue kallikrein/kinin–mediated processes; (2) increasing eNOS expression and activation; and (3) stimulating SIRT1 and SOCS3 expression. Kallistatin via its heparin-binding domain interacts with heparan sulfate proteoglycans, thereby antagonizing the following biological effects: (1) VEGF-mediated angiogenesis and vascular permeability; (2) TNF-α–induced nuclear factor (NF)-κB activation, inflammation, oxidative stress, and apoptosis; (3) HMGB1–induced inflammation; (4) TGF-β–induced fibrosis and endothelial–mesenchymal transition; (4) Wnt-mediated cancer cell proliferation, migration, and invasion; and (5) epidermal growth factor–mediated cancer cell migration and invasion. Thus, kallistatin regulates numerous signaling pathways unrelated to tissue kallikrein. Furthermore, kallistatin modulates a broad range of biological activities, such as blood pressure, angiogenesis, inflammation, oxidative stress, apoptosis, fibrosis, and cancer development.

Kallistatin Is a Novel Vasodilator

The expression and distribution of kallistatin in endothelial and smooth muscle cells of blood vessels implicate its role in vascular function. Rat tissue kallikrein–binding protein, the analogue of human kallistatin, is markedly reduced in the circulation of spontaneously hypertensive rats compared with normotensive rats, suggesting that it may be involved in maintaining normal blood pressure. Kallistatin was identified to be a potent vasodilator, indicating its important role in vascular function. An intravenous bolus injection of purified kallistatin caused a rapid and transient reduction of blood pressure in both normotensive and hypertensive rats. Kallistatin administration also induced vasorelaxation in isolated aortic rings. Neither kallistatin’s blood pressure–lowering effect nor its vasorelaxation ability was abolished by icatibant (Hoe140, a kinin B2 receptor antagonist), indicating that kallistatin-mediated...
vasodilation is unrelated to the tissue kallikrein–kinin system. In addition, kallistatin transgenic mice were found to have significantly lower blood pressure than control littermates. Likewise, expression of human kallistatin after adenovirus-mediated gene delivery caused a prolonged blood pressure reduction in spontaneously hypertensive rats. Conversely, injection of anti-rat kallistatin antibody in normotensive rats induced a time-dependent blood pressure rise for several hours, as determined by both tail-cuff and cannulation methods, further implicating a role for endogenous kallistatin in blood pressure regulation (Figure 1A and 1B). Kallistatin’s active site is essential for its blood pressure–lowering effect because both wild-type kallistatin and heparin-binding site mutant kallistatin, but not active-site mutant kallistatin, induced vasodilation (Figure 1C). Moreover, administration of a NO synthase inhibitor (Nω-nitro-L-arginine methylester, L-NAME) blocked the vasodilating activity of kallistatin, indicating that NO is involved in kallistatin’s ability to reduce blood pressure (Figure 1D). Indeed, kallistatin via its active site increases eNOS expression and activation and, thus, NO production in endothelial cells. Therefore, kallistatin through its active site induces vasorelaxation and decreases blood pressure, in part, through NO formation. These combined findings indicate that kallistatin is a new vasodilator and an endogenous blood pressure–lowering agent.

**Kallistatin Inhibits Angiogenesis and Tumor Progression**

Angiogenesis is an important process in the evolution of inflammatory diseases, which may predispose patients to cancer development. Kallistatin has been shown to exert antitumor activity in breast, colon, stomach, lung, and liver carcinomas by inhibiting angiogenesis, inflammation, and cancer cell proliferation and inducing cancer cell death. A single intramural injection of the kallistatin gene into pre-established breast cancer xenografts in mice resulted in significant suppression of tumor growth and reduction of blood vessel numbers. Systemic injection of lentivirus carrying the human kallistatin gene dramatically decreased cancer metastasis into lungs in association with reduced angiogenesis and inflammation, and also enhanced the survival of tumor-bearing mice. Because VEGF is critical in the development of new blood vessels and tumor growth, it is plausible that kallistatin may interfere with VEGF-mediated processes. Indeed, both wild-type kallistatin and active-site mutant kallistatin, but not the heparin-binding site mutant, inhibited VEGF-induced endothelial cell proliferation, growth, migration, and capillary tube formation. These findings indicate that kallistatin, via its heparin-binding site, interacts with cell surface heparan sulfate proteoglycans, thereby blocking VEGF from binding to its receptor. Kallistatin through the heparin-binding domain also prevented...
TNF-α-induced NF-κB activation and VEGF expression in cultured endothelial cells. Moreover, kallistatin’s active site is essential for stimulating eNOS activation and synthesis and NO formation, which in turn, is capable of inhibiting NF-κB activation. Therefore, kallistatin inhibits angiogenesis not only by interfering with VEGF-mediated effects, but also by down-regulating TNF-α-induced VEGF expression.

In addition to inhibiting angiogenesis, kallistatin’s heparin-binding domain is crucial for antagonizing TNF-α and HMGB1-induced inflammatory gene expression in endothelial cells. Kallistatin via its heparin-binding site blocks TGF-β-induced oxidative stress and miR-21 synthesis in endothelial cells and, thus, endothelial–mesenchymal transition, which is a major contributor to organ fibrosis and tumor progression. Likewise, kallistatin’s heparin-binding site is essential for suppressing Wnt3α signaling and cancer cell proliferation.

Kallistatin antagonizes Wnt3α signaling by forming a complex with the Wnt coreceptor low-density lipoprotein receptor-related protein 6, as demonstrated in retinal epithelial and breast cancer cells, thereby inhibiting Wnt3α-induced tumor cell proliferation, migration, and invasion. Moreover, reduced kallistatin levels are correlated with βII-spectrin, an adapter protein during Wnt signaling in hepatocellular carcinoma. Furthermore, kallistatin prevents the migration and invasion of prostate cancer cells elicited by tissue kallikrein (unpublished observations). Taken together, these findings indicate that kallistatin exerts multifaceted activities in protection against tumor growth and metastasis by inhibiting angiogenesis, inflammation, and cancer cell proliferation, migration, and invasion. As shown in Figure 2, kallistatin’s heparin-binding site is essential for blocking signaling pathways stimulated by VEGF, TNF-α, HMGB1, Wnt, TGF-β, and epidermal growth factor, whereas its active site is key for inhibiting tissue kallikrein–mediated effects.

### Figure 2
Kallistatin, via its heparin-binding site and active site, protects against tumor growth and metastasis by blocking signaling pathways stimulated by vascular endothelial growth factor (VEGF), tumor necrosis factor (TNF-α), high-mobility group box-1 (HMGB1), Wnt, epidermal growth factor (EGF), and transforming growth factor (TGF)-β, and by inhibiting tissue kallikrein–mediated actions.

### Kallistatin Is a Unique Anti-Inflammatory Agent

Inflammation is not only closely linked to cancer, but also to hypertension and organ injury. Recent studies demonstrate that inflammation, oxidative stress, and immunity promote hypertensive end-organ damage. Stimuli, such as angiotensin II and deoxycorticosterone acetate–salt, lead to accumulation of activated immune cells, like T cells and monocytes/macrophages, in the kidney and blood vessels. Moreover, mice lacking NADPH oxidases are protected against angiotensin II–induced vascular inflammation and immune cell infiltration, which clearly establishes a link between oxidative stress and immune activation in hypertension. Numerous studies have indicated that kallistatin inhibits inflammatory responses in both normotensive and hypertensive animal models. Kallistatin gene delivery significantly reduced inflammatory responses and joint swelling in arthritic rats. Kallistatin administration also suppressed inflammatory cell infiltration and prevented complement factor C5a-induced paw edema and vascular leakage in mice. Local delivery of the kallistatin gene into rat heart enhanced cardiac performance and prevented inflammatory cell infiltration after acute myocardial ischemia/reperfusion (I/R). In salt-induced hypertensive rats, kallistatin therapy attenuated vascular and renal damage and reduced oxidative stress and inflammation, whereas depletion of endogenous kallistatin augmented organ injury and accentuated oxidative stress, inflammation, and fibrosis. In addition, kallistatin treatment dramatically improved survival and reduced inflammation, oxidative stress, apoptosis, and organ damage in mice with polymicrobial sepsis and endotoxemia. Mechanistically, kallistatin inhibits vascular inflammation by interacting with the transcription factor Kruppel-like factor 4, leading to increased eNOS expression and NO levels in endothelial cells. Kallistatin’s active site is responsible for upregulating eNOS and SIRT1 expression and, thus, increasing NO production. In normotensive rats, kallistatin via its heparin-binding domain, also ameliorates inflammation by blocking VEGF-induced endothelial cell permeability, as well as TNF-α–induced NF-κB activation, p38 mitogen-activated protein kinase (MAPK) phosphorylation, and inflammatory gene expression in vivo. Likewise, kallistatin’s heparin-binding site was shown to block HMGB1-mediated synthesis of inflammatory genes in endothelial cells. Hence, kallistatin’s heparin-binding domain is vital for inhibiting both early (TNF-α) and late (HMGB1) cytokine-induced inflammatory responses. In cultured macrophages, kallistatin via its active site induced the expression of SOCS3, a negative feedback regulator of inflammation, cancer development, and progression. Thus, kallistatin through its active site and heparin-binding domain exerts anti-inflammatory actions by (1) stimulating eNOS and SIRT1 synthesis and NO formation; (2) increasing SOCS3 expression; (3) antagonizing TNF-α and HMGB1-mediated inflammatory gene expression; and (4) blocking VEGF-induced vascular permeability.
Together, these findings implicate kallistatin to be a novel anti-inflammatory agent that protects against hypertension and end-organ damage by regulating the activation of inflammatory and immune components.

Kallistatin Acts as an Antioxidant to Protect Against Organ Injury

Imbalances between reactive oxygen species formation and endogenous antioxidants can result in oxidative stress. Oxidative stress activates inflammatory pathways and triggers a series of events that lead to organ injury.24 Increased oxidative stress and reduced NO bioavailability are important contributing factors in the pathogenesis of hypertension and cardiovascular and renal diseases.58 Low kallistatin levels are associated with enhanced oxidative stress and organ damage in salt-induced hypertensive rats.51,59 Moreover, serum kallistatin levels are markedly decreased in animal models of hypertension, aortic constriction, streptozotocin-induced diabetes mellitus, renal injury, myocardial I/R, and cerebral I/R (unpublished observations). Kallistatin gene transfer attenuated the pathogenesis of organ damage, inflammation, and apoptosis in conjunction with decreased reactive oxygen species formation and increased eNOS and NO levels in animal models of myocardial I/R, myocardial infarction, and salt-induced hypertension.38,50,51,60 Conversely, depletion of endogenous kallistatin by neutralizing antibody injection compounded cardiovascular and renal damage, which was accompanied by elevated oxidative stress, inflammation, hypertrophy, and fibrosis in deoxycorticosterone acetate–salt hypertensive rats.54 With its antioxidant activity, kallistatin administration protected against CCl4-induced liver fibrosis in rats and lipopolysaccharide-induced acute lung injury in mice.55 Kallistatin also decreased H2O2-mediated oxidative stress and downregulated NAD(P)H oxidase expression in rat corneal epithelium and human hepatic stellate cells.61,62 In cultured endothelial cells, kallistatin via its heparin-binding site antagonized TNF-α and TGF-β–induced NADPH oxidase activity and expression and, thus, reactive oxygen species formation,22,36,38 whereas kallistatin’s active site was crucial for stimulating the synthesis of the antioxidant proteins eNOS, SIRT1, and forkhead box protein O1.22 Furthermore, kallistatin exhibited antioxidant activity in cultured pterygium epithelial cells by increasing expression of superoxide dismutase 2,63 which is mediated by forkhead box protein O1.64 Many of kallistatin’s antioxidant effects occur through NO because it can inhibit NAD(P)H oxidase activity.55 Indeed, kallistatin, via stimulating NO formation, has been shown to reduce superoxide production and NAD(P)H oxidase activity induced by TNF-α, H2O2, or angiotensin II in cultured renal endothelial tubular and mesangial cells, cardiomyocytes, myofibroblasts, and endothelial cells.21,36,38,51,60 The signaling mechanisms by which kallistatin exerts antioxidant activity are presented in Figure 4.

Kallistatin Reduces Vascular Injury by Promoting Endothelial Progenitor Cell Mobility, Viability, and Function

Endothelial progenitor cells (EPCs) are a continuous endogenous source of replenishment for damaged vessels and serve to maintain vascular integrity in response to endothelial injury.66–68 Decreased numbers of circulating EPCs are found in patients with hypertension, chronic renal failure, coronary artery disease, diabetes mellitus, rheumatoid arthritis, and sepsis.66–69,71 EPCs isolated from patients with hypertension and coronary artery disease also display an impaired migratory response.66 Reduced EPC numbers can be attributed to defective mobility and proliferation as well as accelerated apoptosis and senescence. Therefore, augmented mobilization of endogenous EPCs from bone marrow may be an alternative

---

**Diagram Figure 3.** Kallistatin, through its structural elements, exerts anti-inflammatory actions by antagonizing vascular endothelial growth factor (VEGF)–, tumor necrosis factor (TNF)-α–, and high-mobility group box 1 (HMGB1)–mediated effects, and by inducing the expression of endothelial nitric oxide synthase (eNOS), sirtuin 1 (SIRT1), and suppressor of cytokine signaling 3 (SOCS3).

**Diagram Figure 4.** Kallistatin, through its structural elements, exerts antioxidant actions by blocking tumor necrosis factor-α (TNF-α)– and transforming growth factor-β (TGF-β)–induced signaling, and stimulating endothelial nitric oxide synthase (eNOS) and sirtuin 1 (SIRT1) expression. Both eNOS and SIRT1 increase nitric oxide (NO) formation which, in turn, inhibits NAD(P)H oxidase. Moreover, forkhead box protein O1 (FoxO1) activation by SIRT1 subsequently leads to superoxide dismutase 2 (SOD2) expression. Genistein, a tyrosine kinase inhibitor, blocks kallistatin’s effects on eNOS, SIRT1, and FoxO1.
and effective means to promote vascular repair. Indeed, kallistatin gene delivery resulted in elevated circulating EPC number and reduced aortic oxidative stress and glomerular capillary loss in deoxycorticosterone acetate–salt hypertensive rats, whereas kallistatin deficiency further decreased EPC levels and exacerbated vascular oxidative stress and endothelial rarefaction. These findings indicate a novel mechanism of endogenous kallistatin in vascular repair by promoting EPC mobility and function. In vitro, kallistatin stimulated the proliferation, migration, adhesion, and tube formation of EPCs via activation of phosphoinositide 3-kinase-Akt-eNOS and Akt-glycogen synthase kinase-3β signaling pathways, which have both been demonstrated to promote EPC survival and function. Moreover, kallistatin inhibited TNF-α–induced apoptosis in EPCs. These findings indicate that kallistatin plays a protective role in vascular injury by enhancing vascularization and vascular repair through increasing EPC mobility, viability, and function.

**Kallistatin Attenuates Sepsis-Induced Organ Damage and Mortality**

Sepsis is a systemic inflammatory response to infection that can lead to multiorgan dysfunction. Sepsis is a major contributor to morbidity and mortality of intensive care patients and a leading cause of death worldwide. Because numerous signaling cascades are triggered during sepsis, selective blocking of inflammatory mediators is not sufficient to arrest this process. Kallistatin is a negative acute-phase protein because kallistatin expression in the liver is rapidly decreased in rats after endotoxin shock, and circulating kallistatin levels are markedly diminished in patients with sepsis syndrome and liver disease. Interestingly, a kallistatin gene polymorphism is associated with a decreased risk of developing acute kidney injury in patients with septic shock. Studies of animal models of toxic shock have shown that kallistatin is protective against organ injury and lethality. Transgenic mice expressing rat kallistatin are highly resistant to lipopolysaccharide–induced mortality. Moreover, kallistatin gene transfer reduced mortality, bacterial counts, and inflammatory cell numbers, as well as skin and liver damage, in a mouse model of streptococcal infection. In addition, kallistatin treatment in mice with polymicrobial sepsis attenuated lethality, peri- toneal bacterial counts, renal injury and inflammation, and splenic apoptosis. The protective effects of kallistatin in the kidney occurred in conjunction with reduced expression of TNF-α and HMGB1 and increased eNOS synthesis and NO levels. Furthermore, delayed kallistatin administration after the onset of sepsis attenuated mortality and multiorgan injury in mouse models of polymicrobial sepsis and endotoxemia.

Kallistatin treatment inhibited systemic inflammation by reducing circulatory levels of TNF-α and HMGB1 and dramatically upregulating SOCS3 expression in the kidney and lung. Kallistatin gene or protein delivery improved mortality and lung morphology in lipopolysaccharide–induced septic mice and inhibited reactive oxygen species–mediated inflammation and apoptosis in cultured lung epithelial cells. Kallistatin, via its heparin-binding site, blocked TNF-α- and HMGB1–mediated inflammatory gene expression in endothelial cells. Kallistatin’s active site was found to be crucial for stimulating SOCS3 expression in macrophages through activation of a protein kinase C-extracellular signal–regulated kinase (ERK) signaling pathway, illustrating a novel mechanism by which kallistatin protects against sepsis-induced organ damage. These findings indicate that kallistatin administration significantly enhances survival and protects against organ damage during sepsis.

**Kallistatin Regulates Tissue Kallikrein–Mediated Biological Activities**

As a tissue kallikrein inhibitor, kallistatin is capable of regulating its bioavailability in vivo. Kallistatin modulates several biological functions mediated by tissue kallikrein, such as blood pressure, angiogenesis, tumor development, and inflammation infection. For example, purified kallistatin can induce a rapid and transient reduction of blood pressure and relaxation in isolated aortic rings, independent of tissue kallikrein. Kallistatin transgenic mice are hypotensive, and kallistatin gene delivery caused a prolonged blood pressure reduction for 4 weeks in spontaneously hypertensive rats. Intramuscular injection of the kallistatin gene, however, reversed the hypotension of transgenic mice expressing human tissue kallikrein. Therefore, in addition to acting as a potent vasodilator, kallistatin is able to oppose the blood pressure–lowering effect of tissue kallikrein. Notably, because it took 10 days for kallistatin to counter the hypotension in tissue kallikrein transgenic mice, it is possible that kallistatin’s action is attributed to inhibiting renal-mediated sodium retention. Tissue kallikrein is also present in many tumors, such as those of the breast, lung, stomach, pancreas, pituitary, prostate, and uterus. The tissue kallikrein–kinin system is involved in tumor development because icatibant administration suppressed angiogenesis, vascular permeability, and tumor growth in a mouse tumor model. Tissue kallikrein has been shown to promote neovascularization and restore blood flow through kinin B2 receptor-Akt-glycogen synthase kinase-3β (GSK-3β) and VEGF signaling pathways. Kallistatin, however, blocks the tissue kallikrein–induced migration and invasion of prostate cancer cells (unpublished observations). Thus, kallistatin may also regulate tissue kallikrein–mediated tumor metastasis. Finally, tissue kallikrein processes hemagglutinin and thus enhances infection by the influenza virus, whereas kallistatin gene transfer protects against influenza infection in mice by inhibiting tissue kallikrein–mediated hemagglutinin cleavage. This suggests a novel role of kallistatin in suppressing tissue kallikrein’s effect on viral infection. These combined findings indicate that kallistatin modulates different biological actions mediated by tissue kallikrein.

**Double-Edged Roles of Kallistatin**

Kallistatin exerts protection in various biological functions, yet has unique double-edged actions in angiogenesis, apoptosis, and oxidative stress. First, kallistatin has both pro- and antiangiogenic effects. Kallistatin inhibited angiogenesis by blocking VEGF-induced growth and migration of endothelial cells. However, kallistatin enhanced angiogenesis and vascular repair by promoting EPC migration, proliferation, viability, and tube formation. Second, kallistatin has
contradictory activities in apoptosis. Kallistatin administration attenuated apoptosis and cardiovascular and renal injury in rats with myocardial I/R or salt-induced hypertension.\(^{38,50}\) In addition, kallistatin prevented apoptosis and inflammation in mice with polymicrobial sepsis and in cultured lung epithelial cells.\(^{46,50,52}\) Kallistatin blocked TNF-α-mediated apoptosis in cultured endothelial cells and EPCs.\(^{38,72}\) Conversely, kallistatin stimulated apoptosis in cultured retinal endothelial and colorectal and breast cancer cells.\(^{31,32,88}\) Third, kallistatin can act as a pro- and antioxidative agent. Kallistatin suppressed oxidative stress in cultured cardiomyocytes, myofibroblasts, endothelial cells, and EPCs by increasing NO formation, and kallistatin’s effect on oxidative stress was reversed by inhibition of NOS activity.\(^{38,60,72}\) On the other hand, kallistatin treatment exhibited marked bacterial killing activity in mice with streptococcal infection and polymicrobial sepsis, perceivably by increasing oxidative stress in peritoneal neutrophils.\(^{40,81}\) Thus, kallistatin displays double-edged actions in angiogenesis, apoptosis, and reactive oxygen species, depending on the cell types and pathological conditions.

**Kallistatin-Binding Proteins**

Kallistatin modulates several signaling pathways by binding to multiple proteins or extracellular molecules, including (1) tissue kallikrein; (2) heparan sulfate proteoglycans; (3) the Wnt coreceptor low-density lipoprotein receptor-related protein 6; (4) the transcription factor Kruppel-like factor 4; and (5) tyrosine kinase (Table). Through its active site, kallistatin binds to tissue kallikrein and inhibits its enzymatic activity.\(^{1,4}\) Kallistatin via its heparin-binding site prevents VEGF-induced angiogenesis by competing with VEGF binding to heparan sulfate proteoglycans on endothelial cell surfaces, thereby suppressing VEGF-mediated endothelial cell proliferation, migration, and tube formation.\(^{35}\) Similarly, kallistatin antagonizes TNF-α- and HMGB1-mediated inflammatory gene expression via its heparin-binding domain.\(^{36,40}\) In addition, kallistatin’s interaction with Wnt coreceptor low-density lipoprotein receptor-related protein 6 blocks canonical Wnt/β-catenin signaling and the growth and motility of breast cancer cells.\(^{41}\) The transcription factor Kruppel-like factor 4 was identified as a kallistatin-binding protein on the surface of endothelial cells, which can result in enhanced eNOS expression and inhibition of vascular inflammation.\(^{21}\) Moreover, based on Scatchard plot analysis, kallistatin’s vasodilating activity may be attributed to the presence of specific kallistatin-binding sites on aortic membranes.\(^{38}\) Additionally, kallistatin was shown to bind to Müller cells, leading to protection against oxidative stress.\(^{89}\) Furthermore, kallistatin stimulates eNOS and SIRT1 synthesis in endothelial cells and induces SOCS3 expression in macrophages through activation of a cell surface tyrosine kinase because the effect was blocked by genistein, a tyrosine kinase inhibitor.\(^{22,52}\) However, the identity of the cell surface kallistatin receptor remains to be determined. Taken together, these studies demonstrate that kallistatin’s interaction with multiple proteins or molecules accounts for its regulation of various biological activities.

**Kallistatin as a New Biomarker for Human Diseases**

Kallistatin levels are decreased in animal models of hypertension, cardiovascular and renal injury, septic shock, diabetes mellitus, and hepatic neoplasia.\(^{3,51,59,77,90-92}\) Likewise, human kallistatin levels are significantly diminished in numerous diseases. Patients with liver disease, septic syndrome, inflammatory bowel disease, cirrhosis, severe pneumonia, and acute respiratory distress syndrome display decreased levels of kallistatin.\(^{53,78,93-95}\) Plasma kallistatin levels are also reduced in healthy black youths with adiposity and cardiometabolic risk factors, indicating its potential role in metabolic disorders and perhaps the development of obesity.\(^{96}\) Moreover, kallistatin levels are decreased in the vitreous fluids of patients with diabetic retinopathy.\(^{97}\) Furthermore, circulating kallistatin levels are markedly reduced in patients with colon and prostate cancer.\(^{98}\) However, kallistatin levels in the circulation or tissues were shown to be elevated in patients with diabetic vascular complications and rheumatoid joints.\(^{99-101}\) These observations indicate that kallistatin may serve as a novel biomarker for human diseases, such as cardiovascular and metabolic disorders, obesity, and cancer.

**Conclusions**

Kallistatin plays important roles in protection against vascular and multiorgan damage. Kallistatin regulates a wide spectrum of biological activities, such as blood pressure reduction, inhibition of angiogenesis, inflammation, apoptosis, oxidative stress, fibrosis, and cancer growth and invasion. Moreover, kallistatin protects against pathogenesis of vascular and organ injury and tumor progression via its double-edged actions in angiogenesis, apoptosis, and oxidative stress. In addition to regulating the bioavailability of tissue kallikrein, kallistatin by its active site and heparin-binding domain modulates many important signaling pathways. Kallistatin treatment decreases the pathogenesis of hypertension, cardiovascular, renal and lung dysfunction, inflammatory arthritis, sepsis, and cancer progression in numerous animal models. In addition, kallistatin levels in plasma, body fluid, or tissues are markedly reduced in animal models with hypertension, diabetes mellitus, and organ injury. Kallistatin levels are also significantly lower in patients with liver disease, septic syndrome, severe pneumonia, diabetic retinopathy, inflammatory bowel disease, and colon and prostate cancer, as well as in apparently healthy black adolescents with adiposity. Therefore, kallistatin may serve as a new biomarker for the prediction of patient outcomes. Because kallistatin is an endogenous protein, minimal side effects are expected with kallistatin therapy. Thus, kallistatin treatment may potentially be used as a novel therapeutic agent for human diseases.

**Disclosures**

None.

---

**Table. Kallistatin-Binding Proteins**

<table>
<thead>
<tr>
<th>Tissue kallikrein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heparan sulfate proteoglycan</td>
</tr>
<tr>
<td>Low-density lipoprotein-like receptor-related protein (LRP)-6</td>
</tr>
<tr>
<td>Kruppel-like factor 4 (KLF4)</td>
</tr>
<tr>
<td>Tyrosine kinase</td>
</tr>
</tbody>
</table>
References


