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Kallistatin Suppresses Cancer Development by Multi-Factorial Actions

Julie Chao*, **Pengfei Li**, and **Lee Chao**

Department of Biochemistry and Molecular Biology, Medical University of South Carolina, Charleston, South Carolina, U.S.A

Abstract

Kallistatin was first identified in human plasma as a tissue kallikrein-binding protein and a serine proteinase inhibitor. Kallistatin via its two structural elements regulates differential signaling cascades, and thus a wide spectrum of biological functions. Kallistatin's active site is essential for: inhibiting tissue kallikrein's activity; stimulating endothelial nitric oxide synthase and sirtuin 1 expression and activation; and modulating the synthesis of the microRNAs, miR-34a, miR-21 and miR-203. Kallistatin's heparin-binding site is crucial for antagonizing the signaling pathways of vascular endothelial growth factor, tumor necrosis factor-α, Wnt, transforming growth factor-β and epidermal growth factor. Circulating kallistatin levels are markedly reduced in patients with prostate and colon cancer. Kallistatin administration attenuates angiogenesis, inflammation, tumor growth and invasion in animal models and cultured cells. Therefore, tumor progression may be substantially suppressed by kallistatin's pleiotropic activities. In this review, we will discuss the role and mechanisms of kallistatin in the regulation of cancer development.

Keywords

Kallistatin; Cancer; Vascular endothelial growth factor; Angiogenesis; Inflammation; Apoptosis; Hyperoxia

1. Introduction

Chronic inflammation and angiogenesis are critical components of cancer development, as inflammatory cells are actively recruited to the tumor microenvironment [1, 2]. Tumor necrosis factor-α (TNF-α) functions as an inflammatory cytokine, and induces vascular endothelial growth factor (VEGF) expression through paracrine mechanisms [3]. VEGF, in turn, is an essential contributor to the development of angiogenesis and tumor growth [4, 5]. Moreover, transforming growth factor-β (TGF-β) is a potent regulator of tumor metastasis

*Corresponding author at: 173, Ashley Avenue, Charleston, South Carolina, 29425, USA., Tel.: +1 843 792 9927, Fax: +1 843 792 4850, chaoj@musc.edu (Julie Chao).

Conflict of interest

None.

by inducing endothelial-mesenchymal transition (EndMT) in endothelial cells and epithelial-mesenchymal transition (EMT) in epithelial cancer cells [6, 7]. In addition, Wnt signaling is critical for macrophage-induced cancer cell invasion by activating canonical Wnt/β-catenin signal transduction and Wnt-mediated transactivation of epidermal growth factor receptor (EGFR) [8–10]. Furthermore, tissue kallikrein (TK), which is present in endothelial and tumor cells, stimulates angiogenesis via VEGF synthesis and promotes cancer cell migration and invasion [11, 12]. Due to the multiple factors that regulate cancer development, it is a challenge to develop therapeutic regimens that target these diverse signaling pathways.

Kallistatin was discovered in human plasma as a tissue kallikrein-binding protein (KBP) and identified as a unique serine proteinase inhibitor (serpin) in our laboratory [13–17]. Serpins share a general frame of structure, but have diverse functions in the biological processes of apoptosis, inflammation and tumorigenesis [18, 19]. Serpins have been shown to either promote or inhibit tumor growth. For example, high levels of plasminogen activator inhibitor-1 are associated with poor prognosis and predicted adverse outcomes in patients with breast and ovarian cancer [20]. Moreover, α1-antichymotrypsin is up-regulated in multiple cancer types, and the elevated levels are positively correlated with worsening prognosis in patients with breast, lung and gastric cancers [21–23]. Conversely, maspin, an epithelial-specific member of the serpin superfamily, is associated with better prognosis and overall cancer patient survival [24]. Pigment epithelium-derived factor is widely reported to have anti-tumor effects in 17 different types of human cancers [25], and exhibits diverse and significant anti-tumor activities, including inhibition of tumor angiogenesis and metastasis, and enhanced apoptosis in cancer cells [26]. Likewise, circulating kallistatin levels are markedly reduced in patients with prostate and colon cancer, sepsis syndrome and liver disease [27, 28]. Kallistatin administration via local or systemic delivery in animal models retards tumor progression in breast, liver, stomach, colon and lung carcinomas by inhibiting angiogenesis, inflammation and metastasis, thereby implicating a role of kallistatin in tumor suppression [29–34].

2. Kallistatin via its structural domains exerts multiple anti-tumor actions

Kallistatin consists of two functional domains, an active site and a heparin-binding site [35, 36], that regulate differential signaling pathways and a wide spectrum of biological functions. Kallistatin is a unique serpin as its active site consists of Phe-Ser residues at P1-P1’ site, which can be cleaved by TK, thereby forming a covalent complex with TK [35]. Kallistatin via its active site inhibits TK’s enzymatic activity and bioavailability [37, 38], and suppresses TK-induced cancer cell migration and invasion (Fig. 1A & B). Kallistatin’s active site is also the key for stimulating endothelial nitric oxide synthase (eNOS) and sirtuin 1 (SIRT1) expression and activation by interaction with a tyrosine kinase [39]. Up-regulation of eNOS and SIRT1 by kallistatin leads to increased NO levels, a potent anti-inflammatory agent and anti-oxidant [40, 41]. Kallistatin through its active site induces cancer apoptosis by stimulating miR-34a and suppressing miR-21 and miR-203 synthesis in breast cancer cells [42]. Thus, kallistatin’s active site plays a significant role in inhibiting cancer development by modulating the effects mediated by TK, eNOS, miR-34a, miR-21 and miR-203. In addition, kallistatin via its heparin-binding domain binds to heparin sulfate proteoglycans to antagonize the following signaling pathways: VEGF-induced angiogenesis

and vascular permeability [43, 44]; TNF-α- and high mobility group box 1 (HMGB1)-induced NF-κB activation and inflammatory gene expression [44, 45]; TGF-β-mediated oxidative stress and EndMT [39]; Wnt-induced cancer cell proliferation, migration and invasion [42, 46]; and epidermal growth factor (EGF)-induced cancer cell migration and invasion (Fig. 1C & D). Moreover, kallistatin induces cancer cell autophagy by preventing Wnt-mediated inhibition of peroxisome proliferator-activated receptor-γ (PPAR-γ) signaling [42]. Thus, kallistatin, through its functional domains – an active site and a heparin-binding site – regulates multiple signaling pathways to inhibit cancer development (Fig. 2A & B). These findings indicate that kallistatin via its two structural domains exerts multiple cellular functions to suppress cancer development.

3. Kallistatin retards tumor growth and metastasis in tumor-bearing animals

Kallistatin possesses anti-tumor activity as evidenced by inhibiting tumor growth and metastasis in animal models [29–34, 47]. For example, intramural injection of adenovirus carrying the human kallistatin gene into pre-established breast cancer xenografts in nude mice results in significant suppression of tumor growth and reduction of blood vessel numbers [29]. Recombinant kallistatin protein administration by intraperitoneal (IP) injection attenuates tumor growth and intramural neovascularization in either grafted hepatocarcinoma mice or xenografted hepatocarcinoma athymic mice, and reduces VEGF and hypoxia-inducible factor (HIF)-1α expression in hepatocellular carcinoma cells [30]. Likewise, exogenous kallistatin protein treatment by IP injection represses tumor growth and angiogenesis of gastric carcinoma xenografts in mice, and reduces VEGF and HIF-1α synthesis in gastric carcinoma cells [31]. Moreover, adeno-associated virus-mediated kallistatin gene transfer by intramuscular injection reduces angiogenesis and xenographic colon tumor growth in mice [32], and subcutaneous injection of plasmid DNA-mediated kallistatin gene delivery attenuates xenograft lung tumor growth in mice [33]. Furthermore, lentivirus-mediated kallistatin gene delivery by intravenous injection dramatically decreases tumor metastasis into lungs in association with reduced angiogenesis and inflammation, and enhances the survival of tumor-bearing mice [34]. In addition, a combination of local kallistatin gene therapy with meloxicam (a selective cyclooxygenase-2 inhibitor) was shown to have a better therapeutic effect in combating liver cancer in mice by inhibiting tumor growth and angiogenesis, and inducing apoptosis of human hepatocellular carcinoma cells [47]. Thus, addition of extrinsic kallistatin with various strategies by adenovirus, adeno-associated virus, lentivirus, plasmid DNA, or recombinant kallistatin protein, can retard tumor growth and progression in xenografted mice with breast, liver, stomach, colon and lung carcinomas. Importantly, systemic injection of lentivirus carrying human kallistatin cDNA reduces tumor metastasis into lungs and prolongs the survival of mice. Taken together, kallistatin therapy may serve as an effective and promising approach for controlling tumor growth and metastasis.

4. Kallistatin inhibits VEGF-induced angiogenesis

Angiogenesis is an important contributor to cancer progression, and VEGF is critical in the development of new blood vessels and tumor growth [48–51]. Since VEGF is a heparin-binding growth factor, kallistatin via its heparin-binding site competes with VEGF binding

to cell surface heparan sulfate proteoglycans, thereby blocking VEGF-mediated signaling pathways and angiogenesis [43]. Moreover, kallistatin was shown to inhibit gastric carcinoma growth and angiogenesis in conjunction with reduced VEGF expression [31]. Likewise, intraperitoneal injection of kallistatin suppresses tumor growth and angiogenesis in xenografts of hepatocarcinoma in mice, and decreases VEGF synthesis in hepatocarcinoma cells [30]. TNF-α has pro-angiogenic activity via enhancing VEGF expression and secretion in macrophages and tumor cells [52]. However, kallistatin was shown to inhibit TNF-α induced VEGF expression in endothelial cells [53] and breast cancer cells (Fig. 3A & B). Therefore, kallistatin exerts anti-angiogenic actions not only by blocking VEGF-mediated signaling, but also by preventing TNF-α-induced VEGF synthesis.

5. Kallistatin inhibits cancer-related inflammation

TNF-α is produced in a wide variety of tumors and acts as a master switch in establishing an intricate link between inflammation and cancer [49]. Inflammation aids in the proliferation and survival of cancer cells and promotes angiogenesis and metastasis [54]. Activated macrophages are the major source of TNF-α, and it is well recognized that TNF-α functions as a key regulator of the tumor microenvironment [55]. Evidences indicate a critical role of TNF-α in cancer-related inflammation by promoting tumor angiogenesis, proliferation, migration and invasion [56]. Kallistatin levels are markedly reduced in patients and animal models with inflammatory disorders, sepsis syndrome and cancer [27, 28, 57, 58]. A recent study indicated that kallistatin levels in HIV-infected patients are negatively correlated with systemic inflammatory markers, such as IL-6 and high sensitivity C-reactive protein [59]. Kallistatin functions as an anti-inflammatory agent as shown by its ability to suppress: lipopolysaccharide-induced inflammation and lethality in kallistatin transgenic mice; joint swelling and inflammatory response in a rat arthritis model; vascular leakage in a mouse permeability model; inflammatory cell infiltration and TNF-α levels in rat models; and inflammatory responses in septic mice [44, 45, 58, 60–63]. Kallistatin protects against inflammation by antagonizing TNF-α- and HMGB1-mediated nuclear factor (NF)-κB activation and expression of pro-inflammatory genes in cultured endothelial cells [44, 45] and breast cancer cells [53]. Moreover, kallistatin stimulates eNOS expression by interacting with the transcription factor Kruppel-like factor 4, and also increases eNOS activity and NO generation by triggering a phosphoinositide 3-kinase (PI3K)-Akt signaling cascade in endothelial cells and endothelial progenitor cells (EPCs) [40, 41, 64]. Kallistatin not only stimulates eNOS expression [39], but also prevents TNF-α-mediated inhibition of eNOS synthesis in endothelial cells (unpublished results). NO production can inhibit the expression of cell adhesion molecules by preventing activation of the pro-inflammatory transcription factor NF-κB [65]. Therefore, kallistatin exerts anti-inflammatory actions via regulating differential signaling pathways, namely: 1) preventing TNF-α- and HMGB1-induced inflammatory gene expression; 2) stimulating eNOS synthesis and activation, and NO formation; 3) reversing TNF-α mediated suppression of eNOS synthesis; and 4) reducing VEGF-induced vascular permeability. Thus, kallistatin is a unique anti-inflammatory agent in protection against cancer development.

6. Kallistatin inhibits TGF-β-induced EndMT and EMT

As metastasis causes 90% of cancer patient mortality, understanding the initial step of metastasis is critical to the future development of novel strategies to prevent the spread of cancer. EndMT and EMT play pivotal roles in fibrosis and tumor metastasis [66–68]. TGF-β is the most potent inducer of EndMT and EMT, as TGF-β signaling is involved in controlling endothelial or epithelial plasticity by eliciting their transition to a mesenchymal state [68–70]. miR-21 is an important player in organ fibrosis and tumor invasion, and its expression levels rise to a significant extent during EndMT [70, 71]. TGF-β-induced EndMT is partly regulated by miR-21, as blockade of miR-21 was found to prevent EndMT [6, 39]. Moreover, reactive oxygen species (ROS) production leads to increased miR-21 synthesis [72]. Kallistatin treatment exerts beneficial effects on cardiac and renal fibrosis by suppressing TGF-β synthesis and oxidative stress in animal models, and antagonizing TGF-β-induced collagen synthesis in cardiac myofibroblasts [73, 74]. In endothelial cells, kallistatin via its heparin-binding site antagonizes TGF-β-induced miR-21 synthesis and ROS formation, while its active site is crucial for stimulating the synthesis of antioxidant genes, including eNOS, SIRT1 and forkhead box O1 (FoxO1) [39]. Furthermore, kallistatin suppresses TGF-β-induced EMT in epithelial MCF-7 breast cancer cells, as identified by increased E-cadherin and reduced snail-1, fibronectin and vimentin synthesis (unpublished results). In addition to TGF-β, EMT is also triggered by other secreted growth factors, such as Wnt, TNF-α and EGF [75–78]. Therefore, kallistatin could protect against cancer invasion, in part, by suppressing EndMT and EMT.

7. Kallistatin inhibits cancer cell proliferation, migration and invasion

Kallistatin is capable of inhibiting the proliferation, migration and invasion of cancer cells induced by Wnt, TGF-β and EGF [39, 43, 46]. Wnt signaling is critical for macrophage-induced cancer cell invasion by activating the canonical Wnt/β-catenin signaling pathway and Wnt-mediated transactivation of EGFR [8, 9]. Kallistatin via its heparin-binding site suppresses Wnt3a-induced proliferation, migration and invasion of breast cancer cells [42, 46]. Kallistatin antagonizes the Wnt3a signaling pathway by forming a complex with Wnt co-receptor low-density lipoprotein receptor-related protein 6 (LRP6) in breast cancer and retinal epithelial cells [46, 61]. As TNF-α has been shown to promote Wnt signalling in gastric tumor cells [79], kallistatin may also block TNF-α-mediated Wnt/β-catenin signaling. In hepatocellular carcinoma, decreased β-II spectrin (SPTBN1) is associated with reduced kallistatin levels, leading to elevated Wnt signal transduction [80]. Kallistatin administration inhibits colon tumor growth by inhibiting angiogenesis and tumor cell proliferation [32]. Furthermore, kallistatin could retard cancer cell invasion by blocking TGF-β-mediated EndMT [39] and EMT. In addition, kallistatin via its heparin-binding site antagonizes EGF-induced migration and invasion of prostate cancer cells (Fig. 1C & D). Thus, kallistatin through its heparin-binding site inhibits the growth, migration and invasion of cancer cells mediated by Wnt3a, TGF-β and EGF.

8. Kallistatin inhibits TK/kinin-induced cancer development

TK is a serine proteinase that cleaves low molecular weight kininogen substrate to release kinin peptides [81]. Kinins and their receptors are well-known inducers of the pro-inflammatory response [82, 83]. TK is present in many tumors, such as those of the breast, lung, stomach, pancreas, pituitary, prostate, and uterus [84]. The kallikrein-kinin system is involved in tumor development, as administration of icatibant (a kinin B2 receptor antagonist) suppressed angiogenesis, vascular permeability and tumor growth in mice [85]. Kinin B2 receptor signaling facilitates cancer progression, as tumor-associated angiogenesis and growth are markedly suppressed in rodent models genetically deficient in kininogen and kinin B2 receptor [86]. Moreover, a selective TK inhibitor or a kinin B2 receptor antagonist ameliorates vascular permeability and inflammatory cell infiltration in the intestine and lung following ischemia/reperfusion injury [87, 88]. TK gene transfer improves cardiac function and promotes neovascularization by increasing Akt and glycogen synthase kinase-3β (GSK-3β) phosphorylation, leading to increased VEGF and VEGF receptor-2 expression in ischemic myocardium and cultured endothelial cells [89]. Likewise, kinin stimulates endothelial cell proliferation and capillary tube formation through transactivation of VEGF receptor-2 by the kinin B2 receptor [90, 91]. Thus, TK and kinin promote neovascularization and restore blood flow through kinin B2 receptor-Akt-GSK-3β and VEGF signaling pathways [89]. As an endogenous TK inhibitor, kallistatin is capable of blocking TK-induced migration and invasion of prostate cancer cells (Fig. 1A & B). These findings indicate a potential role of kallistatin in controlling TK/kinin-mediated angiogenesis, tumor growth and invasion.

9. Kallistatin induces cancer cell apoptosis and autophagy

Numerous evidences indicate that microRNAs are key players in cancer biology, acting as oncogenes or tumor suppressor genes [92]. Among miRNAs, miR-34a is a critical tumor suppressor in many types of cancers [93–96]. In breast cancer, miR-34a expression is decreased [96], and overexpression of miR-34a induces apoptosis and suppresses cell proliferation and migration [96–98]. On the other hand, miR-21 is a well-recognized tumor inducer, as elevation of miR-21 in breast cancer patients is associated with poor survival [99]. An anti-miR-21 inhibitor has been shown to inhibit levels of the survival protein Bcl-2 in breast cancer [100]. miR-203 may also play a role in cancer progression as it is overexpressed in human breast cancer, while miR-203 knockdown sensitizes apoptotic cell death in MCF-7 breast cancer cells [101]. Moreover, recent studies have shown that autophagy can suppress tumor cell growth, which is a promising strategy for the treatment of breast cancer [102, 103].

Kallistatin was found to induce apoptosis in breast cancer cells by stimulating miR-34a and inhibiting miR-21 and miR-203 synthesis in MDA-MB-231 breast cancer cells [42]. In addition, kallistatin promotes breast cancer cell autophagy, which was mediated by preventing Wnt-mediated inhibition of PPAR-γ signaling [42]. Furthermore, kallistatin (SERPINA3K) induces apoptotic cell death in cultured colorectal cancer cells via activating the PPAR-γ-Fas-FasL signaling pathway [104]. Kallistatin's active site plays a key role in modulating miR-34a, miR-21 and miR-203 synthesis, while its heparin-binding site is

essential for blocking Wnt-PPAR-γ signaling in breast cancer cells [42]. Therefore, kallistatin can suppress tumor development by inducing cancer cell apoptosis and autophagy.

10. Role of kallistatin in hyperoxia-mediated cancer cell death

Hyperoxia can retard growth and induce apoptosis in rat mammary tumors [105]. Likewise, kallistatin inhibits tumor growth and metastasis in tumor-bearing mice [29, 34], and induces apoptosis and autophagy in breast cancer cells [42]. Hyperoxia treatment or H_2O_2 incubation markedly increases kallistatin expression in both MCF-7 and MDA-MB-231 breast cancer cells (Fig. 4A–D). Moreover, the antioxidant *N*-acetyl-L-cysteine (NAC) blocks H_2O_2-induced kallistatin expression (Fig. 4C & D). These findings indicate that hyperoxia and H_2O_2, through ROS formation, induce kallistatin expression in cancer cells. As with kallistatin [42], hyperoxia treatment also induces the expression of apoptosis and autophagic markers, such as BAX, ATG5 and Beclin-1, in breast cancer cells (unpublished results). These findings implicate a potential role of kallistatin in mediating the effect of hyperoxia on cancer cell death.

11. Conclusion

Kallistatin exerts multi-factorial activities in retarding tumor progression. Cancer cells are able to switch a normal microenvironment to one that supports tumor growth and metastasis by inducing angiogenesis and inflammation. Kallistatin is an effective inhibitor of angiogenesis and inflammation. In murine models of tumor xenografts, kallistatin treatment inhibits the development of diverse cancers, including breast, liver, colon, lung and gastric carcinoma [29–34, 47]. Moreover, systemic delivery of the kallistatin gene dramatically inhibits experimental lung metastasis, inflammation and angiogenesis in tumor-bearing mice [34]. Kallistatin through its two functional domains regulates differential signaling pathways, thus protects against tumor growth and metastasis. Kallistatin's active site is crucial for inhibiting TK-mediated cancer progression; suppressing inflammation by increasing eNOS and SIRT1 levels; and inducing cancer cell apoptosis by stimulating miR-34a and reducing miR-21 and miR-203 synthesis [42]. Kallistatin via its heparin-binding site antagonizes VEGF-induced angiogenesis; TNF-α- and HMGB1-induced inflammation; TGF-β-mediated EndMT and EMT; Wnt3a-, TGF-β- and EGF-induced cancer cell migration and invasion; and Wnt-PPARγ-mediated cancer cell autophagy [39, 42–46]. Therefore, kallistatin exerts pleiotropic activities to inhibit tumor progression. Plasma kallistatin levels are significantly reduced in patients with prostate and colon cancer [28]. Recent studies highlight the potential of kallistatin as a biomarker for the diagnosis of liver cirrhosis and an independent prognostic indicator for colorectal cancer [106, 107]. Therefore, it is possible that kallistatin's role as a biomarker can also be applied to diverse human cancers. Moreover, kallistatin-based therapy may constitute a significant advancement in cancer treatment due to its multiple anti-tumor actions. The potential role of kallistatin therapy in humans requires further investigation to warrant clinical studies of kallistatin in cancer treatment.

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Highlights

- Kallistatin treatment inhibits tumor growth and metastasis in animal models
- Kallistatin inhibits tumor angiogenesis and cancer-related inflammation
- Kallistatin inhibits EndMT and EMT
- Kallistatin inhibits cancer cell proliferation, migration and invasion
- Kallistatin inhibits tissue kallikrein-mediated cancer cell invasion
- Kallistatin induces cancer cell apoptosis and autophagy

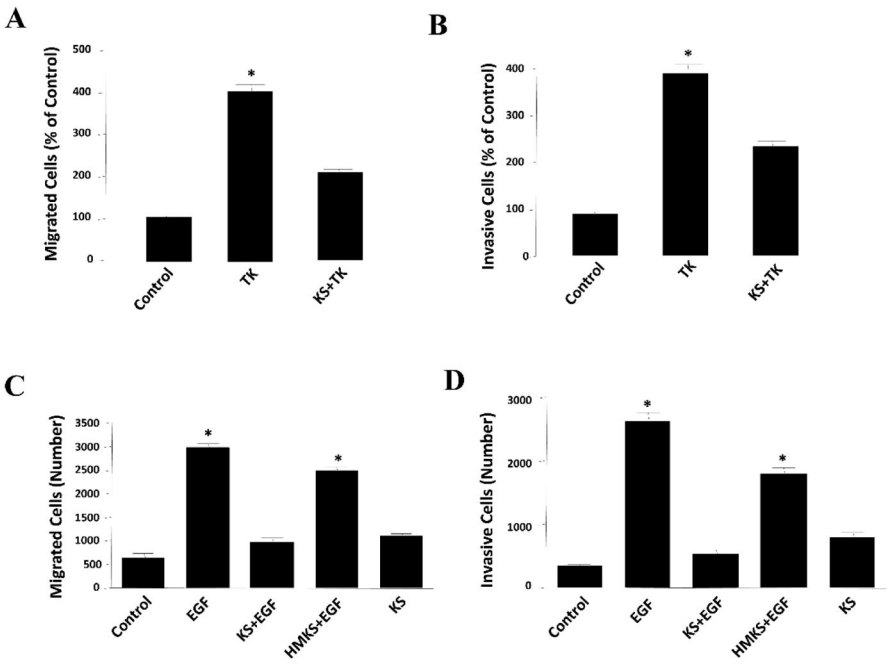

Figure 1.

Kallistatin inhibits the migration and invasion of prostate cancer cells. Kallistatin (1 μM) prevented tissue kallikrein (TK, 0.1 μM)-induced (A) migration and (B) invasion of DU-145 prostate cancer cells. Wild-type kallistatin (KS, 1 μM), but not heparin-binding site mutant kallistatin (HMKS, 1 μM), antagonized epidermal growth factor (EGF, 1 ng/ml)-induced (C) migration and (D) invasion of DU-145 prostate cancer cells, indicating that kallistatin's effects are mediated via its heparin-binding domain. n=3, *P<0.05 vs. other groups.

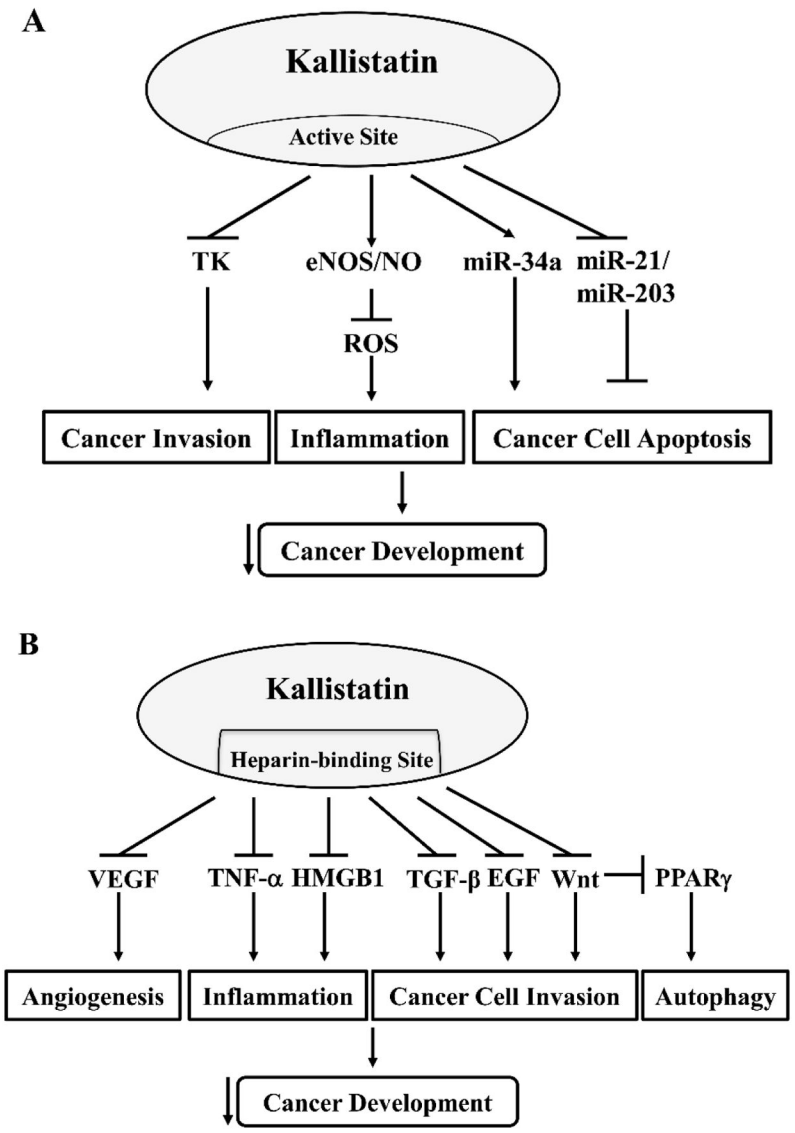

Figure 2.

Kallistatin's active site and heparin-binding site play differential roles in blocking cancer development. (A). Kallistatin's active site is key for modulating the effects induced by tissue kallikrein (TK), endothelial nitric oxide synthase (eNOS), nitric oxide (NO), miR-34a, miR-21 and miR-203. (B). Kallistatin's heparin-binding site is essential for inhibiting the effects mediated by vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α), high mobility group box 1 (HMGB1), transforming growth factor-β (TGF-β), epidermal growth factor (EGF) and Wnt.

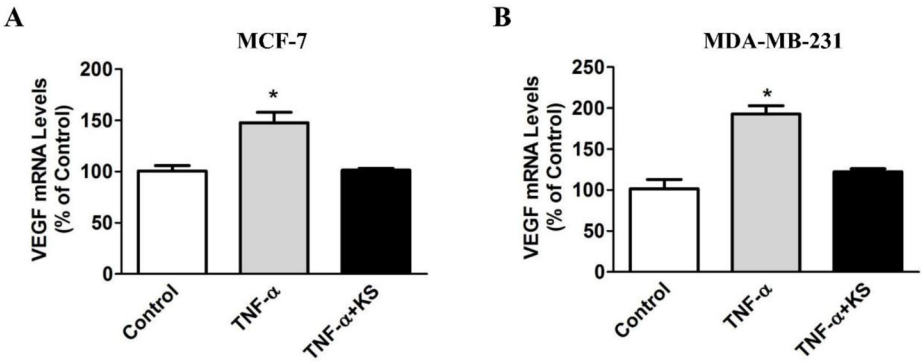

Figure 3.

Kallistatin blocks tumor necrosis factor-α (TNF-α)-induced vascular endothelial growth factor (VEGF) expression in breast cancer cells. Cells were pre-treated with kallistatin (KS, 1 μM) for 30 min, followed by incubation with TNF-α (2 ng/ml) for 12 hr. Kallistatin significantly inhibited TNF-α induced VEGF expression in (A) MCF-7 and (B) MDA-MB-231 breast cancer cells. n=3, **P*<0.05 vs. other groups.

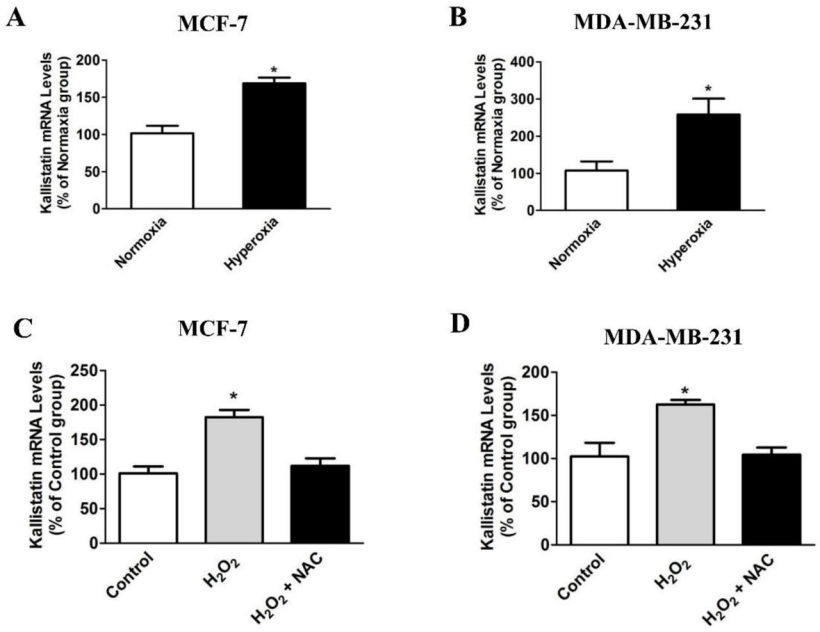

Figure 4.

Hyperoxia or H_2O_2 treatment increases kallistatin expression in breast cancer cells. Hyperoxia (95% O_2 and 5% CO_2) treatment for 18 hr significantly increased kallistatin synthesis in (A) MCF-7 and (B) MDA-MB-231 breast cancer cells. H_2O_2 incubation (30 μM) for 12 hr also increased kallistatin synthesis in (C) MCF-7 and (D) MDA-MB-231 breast cancer cells. Kallistatin expression was blocked by the antioxidant *N*-acetyl-L-cysteine (NAC, 2 mM). n=3, **P*<0.05 vs. other groups.