Renal Biomarker and Angiostatic Mediator? Cystatin C as a Negative Regulator of Vascular Endothelial Cell Homeostasis and Angiogenesis

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Cystatin C, a member of the human cystatin type 2 superfamily, is a small molecular weight (~13 kDa) protein that appears to be produced and constitutively secreted by virtually all nucleated cells in the human organism.1,2 Mechanistically, cystatin C is a potent competitive inhibitor of cysteine proteases, such as cathepsins B, H, and L, suggesting that it mainly controls extracellular protease activity.2 Moreover, extracellular cystatin C has been shown to modulate lysosomal protein turnover after cellular internalization via endocytosis, thereby indicating that secreted extracellular cystatin C also has the ability to modulate target tissue homeostasis after cellular reuptake in vivo.3,4 Cystatin C has gained broad attention primarily as a reliable marker of kidney function in patients with chronic kidney disease.5 Moreover, and interestingly, elevated plasma levels of cystatin C have been associated with the prevalence of cardiovascular risk factors, arterial stiffness, coronary artery disease, and cerebrovascular complications.6 In addition, cystatin C may represent an independent predictor of cardiovascular events and mortality in individuals with or without overt chronic kidney disease.6,7 Although it has been suggested that the predictive value of cystatin C in these clinical settings may be exclusively attributed to related changes in kidney function,5 these clinical observations may also indicate a direct role of cystatin C in the pathophysiology of vascular dysfunction and cardiovascular disease independent of its role as a marker of the glomerular filtration rate.

Nevertheless, results from experimental mouse models involving animals with global cystatin C deletion have indicated rather beneficial effects of cystatin C in the pathogenesis of atherosclerotic vascular disease.8 Global deletion of cystatin C, for instance, may increase the atherosclerotic plaque burden in apolipoprotein E–deficient mice, promote the formation of abdominal aortic aneurysms, and favor atherosclerotic plaque angiogenesis caused by the resulting overactivity of cysteine proteases in the arterial vessel wall.9–11 In addition, cystatin C–related neuroprotective effects have been observed in preclinical disease models.12,13 Thus, the link among elevated cystatin C plasma levels, vascular dysfunction, atherogenesis, and cardiovascular risk in humans is still poorly understood, and further experimental studies are needed to clarify the potential role of cystatin C in cardiovascular disease initiation and progression.

In a first step, using a gerbil animal model, the authors examined the association between cystatin C serum levels and the incidence of vascular abnormalities of the Circle of Willis, a circulatory anastomosis of cerebral arteries located at the base of the brain. The authors observed that serum levels of cystatin C were higher in animals that developed incomplete variants of the Circle of Willis, results that may indicate a role of cystatin C in vascular development. In this context, expression analyses confirmed that cystatin C expression peaks during late embryonal development, whereas its expression remains constant at considerably lower levels in heart and brain tissue after birth. These results led the authors to hypothesize that secreted cystatin C inhibits the de novo formation of blood vessels through direct interaction with vascular endothelial cells. To test this hypothesis, the authors...
conducted a number of in vitro experiments involving primary human and rat endothelial cells. In these experiments, the authors demonstrated that exogenous cystatin C reduced, whereas neutralization of endogenously secreted cystatin C increased spontaneous and VEGFA (vascular endothelial growth factor A)-related endothelial cell proliferation, migration, permeability, and survival in vitro. Moreover, the results were partly confirmed by RNA interference experiments. In these experiments, short hairpin RNA–induced downregulation of cystatin C expression increased migration and proliferation of both endothelial cell types under investigation, whereas overexpression of cystatin C was ineffective in this context. In addition, the authors were able to show that, again, exogenous cystatin C inhibited, whereas antagonism of secreted cystatin C enhanced, spontaneous and VEGFA-induced neovascularization in the Chicken chorioallantoic Membrane (CAM) model of angiogenesis in vivo. Last, neutralization of VEGFA in the growth medium of cultured endothelial cells increased cystatin C mRNA expression and protein content as well as cystatin C release from these cells. Consequently, these findings suggest that cystatin C is a negative regulator of angiogenesis and endothelial cell homeostasis both in vitro and in vivo.

The study contributes several valuable findings to the field of cystatin C and angiogenesis research; however, it also has certain limitations. An obvious limitation is that the present study cannot provide a conclusive explanation for its key observation, namely, the angiostatic properties of cystatin C. In an attempt to fill this mechanistic gap, the authors showed that cystatin C induced an increase in the vascular endothelial p53 protein content—a proto-oncogene with known antiproliferative and angiostatic properties.15 Moreover, cystatin C also induced the protein content of the calcium-dependent cysteine protease calpain 10, an atypical protease that has been implicated in the pathophysiology of diabetes mellitus.16,17 In contrast, known cystatin C targets, such as proangiogenic cathepsin B, H, or L, were not examined. Further studies are needed to clarify how cystatin C favors vascular endothelial protein accumulation of p53 and whether the angiostatic potential of cystatin C depends on inhibition of cathepsins or other cysteine proteases. Another limitation of the study is the uncertain specificity of the blocking peptides used to neutralize exogenous cystatin C or VEGFA. Although dose dependency could be demonstrated (eg, for VEGFA antagonism-related expression and release of cystatin C from vascular endothelial cells), future studies are needed to confirm these findings with additional specific VEGFA-neutralizing antibodies as well as VEGF receptor antagonists that selectively disrupt endothelial VEGFA signaling.

Despite these limitations, the study of Li and colleagues uncovers an inhibitory role of cystatin C in the process of angiogenesis and indicates that VEGFA may regulate cystatin C release from the vascular endothelium. Several questions, including how cystatin C exerts its angiostatic effects, need to be addressed in further studies involving systematic cystatin C plasma-level variations or postnatal tissue-specific cystatin C deletion in experimental models of cardiovascular disease and regeneration. In addition, future research will have to clarify whether cystatin C release may contribute to the considerable spectrum of cardiovascular side effects induced by VEGFA-blocking agents and whether therapeutic reduction of cystatin C plasma levels may represent a useful strategy to improve vascular dysfunction in cardiovascular high-risk patients.

Disclosures
None.

References
1. Abrahamsson M, Olafsson I, Palsdottir A, Ulsvåg M, Lundwall A, Jensson O, Grubb A. Structure and expression of the human cystatin C gene. Biochem J. 1990;268:287–294.
2. Turk V, Stoka V, Turk D. Cystatins: biochemical and structural properties, and medical relevance. Front Biosci. 2008;13:5406–5420.
3. Ekström U, Wallin H, Lorenzo J, Holmqvist B, Abrahamsson M, Avlidas FX. Internalization of cystatin C in human cell lines. FEBS J. 2008;275:4571–4582.
4. Mathews PM, Levy E. Cystatin C in aging and in Alzheimer’s disease. Ageing Res Rev. 2016;32:38–50.
5. Shlipak MG, Mattes MD, Peralta CA. Update on cystatin C: incorporation into clinical practice. Am J Kidney Dis. 2013;62:595–603.
6. Angélidis C, Deffereos S, Giannopoulou G, Anatomolotakis N, Bours G, Hatzis G, Panagopoulou V, Pyrgakis V, Cleman MW. Cystatin C: an emerging biomarker in cardiovascular disease. Curr Top Med Chem. 2013;13:164–179.
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