The initiation, progression, and complications of atherosclerosis are mediated by a complex interplay of hematopoietic cells, vascular cells, and the extracellular matrix in response to metabolic (e.g., hyperlipidemia, diabetes mellitus) and environmental (e.g., smoking) injury. Antiatherosclerotic therapies are currently limited to statins and their lipid-lowering and pleiotropic anti-inflammatory effects, to angiотensin-converting enzyme inhibitors and their local effects on vascular cell proliferation and plaque progression independently of blood pressure lowering, and to antplatelet agents and their role in preventing the thrombotic complications of plaque rupture and superficial endothelial cell (EC) erosion. However, although these therapies have been shown to reduce event rates by ≈20% to 40% in randomized, placebo-controlled clinical trials, absolute event rates remain high. For example, recurrent ischemic events after index acute coronary syndrome presentation occur in >20% of patients by 36 months despite optimal medical therapy and revascularization. This failure of drug and device therapies patients by 36 months despite optimal medical therapy and revascularization. However, although these therapies have been shown to reduce event rates by ≈20% to 40% in randomized, placebo-controlled clinical trials, absolute event rates remain high. For example, recurrent ischemic events after index acute coronary syndrome presentation occur in >20% of patients by 36 months despite optimal medical therapy and revascularization. This failure of drug and device therapies represents an enormous unmet clinical need. In contrast to effective disease-modifying therapies in psoriasis, rheumatoid arthritis, and multiple sclerosis, targeted antiatherosclerotic therapies have remained elusive. In this issue of Circulation, Miyazaki and colleagues5 have identified m-calpain (calpain 2 and its regulatory domain, calpain 4) as a novel protease target for the modulation of atherogenesis.

After 7 transmembrane superfamily receptors (G protein–coupled receptors), proteases are one of the most attractive pharmacological targets. The US market for protease–targeted drugs in 2009 was approximately $11 billion, with the major focus on cardiovascular (hypertension: angiotensin-converting enzyme and renin; thrombosis: thrombin and factor Xa), inflammatory (caspase 1), infectious (HIV protease), and metabolic (diabetes mellitus; dipeptidyl peptidase IV) diseases. Proteases using different mechanisms of substrate hydrolysis (e.g., serine, cysteine, threonine, aspartic proteases, metalloproteases) modulate virtually all aspects of atherosclerotic lesion formation and subsequent plaque activation and thrombosis secondary to direct effects on extracellular matrix degradation; vascular cell apoptosis, proliferation, and migration; and initiation of coagulation (the Figure). The blood coagulation cascade described by Davie and Ratnoff7 and MacFarland8 is the prototypical example of protease signaling through the activation of zymogens by limited proteolysis. Final activation of this cascade results in the generation of the serine protease thrombin, one of the most intensely targeted proteases in medicine with indirect (unfractionated and low-molecular-weight heparins promoting antithrombin III binding) and direct (bivalirudin, argatroban, dabigatran) mechanisms of pharmacological inhibition. Importantly, there is precedence for 1 protease having far-reaching, multifunctional roles in atherosclerosis. Leukocyte-derived myeloperoxidase generates HOCl, which promotes the formation of oxidized low-density lipoprotein, dysfunctional high-density lipoprotein, and impaired EC function, in part by altering the bioavailability of nitric oxide. Myeloperoxidase may also contribute directly to EC desquamation, which is associated with thrombosis by promoting EC apoptosis and detachment.

m-Calpain is a member of the calpain family of calcium-activated, nonlysosomal cysteine protease active at neutral pH. Calpains have ubiquitous and tissue-specific isoforms that exhibit broad substrate specificity and regulate cellular migration, proliferation, and apoptosis. Importantly, calpain activity in vivo is tightly regulated by its endogenous inhibitor calpastatin, and in this regard, it is similar to other protease systems (i.e., serpin inhibitors of serine proteases and tissue inhibitors of metalloproteinasises). Miyazaki and colleagues5 investigated the role of m-calpain in proteolytic disorganization of VE-cadherin and the subsequent progression of atherosclerosis. Increased expression of m-calpain was observed in aortic ECs in atherosclerotic lesions, and proteolysis of VE-cadherin was shown in aortic ECs in ldlr<sup>−/−</sup> and apoE<sup>−/−</sup> mice. Long-term administration of the nonspecific calpain inhibitors N-acetyl-leu-leu-methional and calpeptin into these mice attenuated atherosclerotic lesion development and vascular inflammation independently of lipids. Specificity for m-calpain was inferred by the finding that in vivo transfection of m-calpain siRNA to ldlr<sup>−/−</sup> mice prevented disorganization of VE-cadherin and proatherogenic hyperpermeability in aortic ECs. Interestingly, treatment of cultured ECs with oxidized low-density lipoprotein, lysophosphatidylcholine, or low-density lipoprotein pretreated with secreted phospholipase A2 led to selective induction of m-calpain but not μ-calpain (calpain 1). The authors conclude that m-calpain directly cleaves a juxtamembrane region of...
VE-cadherin, resulting in dissociation of β-catenin from the VE-cadherin complex, disorganization of adherence junctions, and hyperpermeability in ECs, thereby promoting extravasation of inflammatory cells or proatherogenic macromolecules into the vascular wall.

The proposed strategy of selective inhibition of m-calpain for the prevention/treatment of atherosclerotic disease is intriguing and raises important questions. First, is selective inhibition of m-calpain achievable in vivo? No inhibitor is completely selective for a single protease target. The calpain inhibitors (N-acetyl-leu-leu-methional and calpeptin) used in this study lack specificity among cysteine proteases with, for example, significant inhibitory activity against calpain 1, calpain 2, and cathepsins B, K, L, and S.14 Cathepsins K15 and S16 play critical roles in atherogenesis, possibly confounding the interpretation of N-acetyl-leu-leu-methional and calpeptin experiments. Recent advances in elucidating the tertiary structures of calpain 2 and its regulatory domain calpain 4 have resulted in the development of a new class of calpain inhibitors that interact with domains outside the catalytic site and may provide greater specificity and an improved safety profile secondary to reversible inhibition.17 Second, what is the most appropriate timing of m-calpain inhibition? The role of m-calpain in disrupting endothelial barrier cell function suggests that inhibitors will need to be administered early in the disease process, focusing primarily on asymptomatic individuals with risk factors alone or subclinical disease. Third, is the induction of m-calpain also accompanied by a reduction in the endogenous inhibitor calpastatin, which further increases proteolytic activity? Fourth, are other mechanisms besides altered EC barrier function responsible for the proatherogenic effects of m-calpain? There is experimental evidence that calpains also promote vascular cell proliferation18 and platelet activation.19 In addition, the dissociation of β-catenin from VE-cadherin provides a plausible mechanism linking proteolysis and altered gene expression. Interestingly, β-catenin activity has been identified in atherosclerosis-prone regions of the arterial tree and shown, through interactions with the T-cell–specific transcription factor complex, to alter the expression of targets (eg, fibronectin, monocyte chemoattractant protein-1, interleukin-8, bone morphological protein-4) that promote lesion formation.20 Finally, are the calpain 2 genes (CAPN2 and its regulatory domain, CAPN4) disease-susceptibility genes for atherosclerosis? Prior studies demonstrating the association of calpain 10 gene haplotypes with both diabetes mellitus21 and carotid intima-media thickness22 suggest that this is likely worthy of consideration. Only a fraction (~50) of the estimated 500 to 600 proteases in the human genome23,24 are validated pharmacological targets or are currently considered potential targets.6 Therefore, there is great interest in both the identification of novel proteases and their endogenous substrates using ad-
vanced proteomics and genetic (ie, RNA interference) and in silico biology approaches. Miyazaki and coworkers used a candidate molecule approach by simply staining for protease expression in atherosclerotic versus nondiseased arterial segments. It is likely that continued focused and unbiased protease efforts will yield new biological insights that can be exploited for therapeutic gain.

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References
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