A Disintegrin and Metalloprotease 17 Mediates Neointimal Hyperplasia in Vascularu

Akira Takaguri, Keita Kimura, Akinari Hinoki, Allison M. Bourne, Michael V. Autieri, Satoru Eguchi

Abstract—The requirement of a metalloprotease, a disintegrin and metalloprotease 17 (ADAM17) for the growth of cultured vascular smooth muscle cells has been demonstrated in vitro. However, whether this metalloprotease is responsible for vascular remodeling in vivo remains unanswered. Rat carotid arteries were analyzed 2 weeks after a balloon angioplasty. The neointimal cells were strongly positive for ADAM17 immunostaining. Marked inhibition of intimal hyperplasia was observed in a dominant-negative ADAM17 adenovirus-treated carotid artery. Proliferating cell nuclear antigen-positive cells and phospho-epidermal growth factor receptor-positive cells in the neointima were reduced by dominant-negative ADAM17 as well. In contrast, the neointima formation, proliferating cell nuclear antigen-positive cells, and phospho-epidermal growth factor receptor-positive cells were markedly enhanced by wild-type ADAM17 adenovirus. In conclusion, ADAM17 activation is involved in epidermal growth factor receptor activation and subsequent neointimal hyperplasia after vascular injury. ADAM17 could be a novel therapeutic target for pathophysiological vascular remodeling. (Hypertension. 2011;57:841-845.) * Online Data Supplement

Key Words: ADAM proteins * tumor necrosis factor-α convertase * angioplasty * vascular intima * epidermal growth factor receptor

A disintegrin and metalloproteases (ADAMs) are membrane-anchored metalloproteases implicated in the ectodomain shedding of cell surface proteins, including the ligands for epidermal growth factor (EGF) receptors (EGFRs)/ErbBs. It has been well documented that the transactivation of the EGFR plays critical roles for many cellular functions in the cardiovascular system, such as hypertrophy, proliferation, and migration mediated through multiple G protein-coupled receptors.

We have demonstrated that ADAM17 is responsible for the EGFR transactivation and subsequent hypertrophy by angiotensin II in cultured vascular smooth muscle cells (VSMCs). However, in vivo evidence for a role of ADAM17 in mediating cardiovascular diseases remains limited. Here, we hypothesized that targeted inactivation of ADAM17 may reduce proliferating vascular remodeling. To test the hypothesis, we have used a model of arterial hyperplasia in response to angioplasty together with adenoviral gene manipulation of ADAM17.

Materials and Methods

Balloon Angioplasty and Adenoviral Gene Transfer

Left common carotid artery balloon angioplasty was performed in male Sprague-Dawley rats (Charles River Breeding Laboratory), as reported previously. Adenoviral vectors encoding wild-type mouse ADAM17 (wtADAM17) and a catalytically inactive/dominant-negative mouse ADAM17 mutant, E406A (dnADAM17), were created using the pCMV expression vectors as the template. The wtADAM17 and dnADAM17 sequences were amplified by PCR and ligated into the pAdTrack-CMV vector at the BgIII/NorI site. The fragment containing the wtADAM17 or dnADAM17 with green fluorescent protein (GFP) sequence was cloned into pENTR4 vector by the TOPO cloning reaction (Invitrogen) and then cloned into the pAd/CMV/DEST vector by a reaction with LR Clonase II (Invitrogen). The adenovirus titers were determined by Adeno-X Rapid Titer kit (BD Biosciences). Subsequently, 100 μL of the adenovirus encoding wtADAM17, dnADAM17, or control GFP (2×10⁹ pfu/mL) was delivered to the injured artery. We have confirmed protein expression of an adenoviral-encoded gene in medial VSMCs and neointimal cells 14 days after the delivery. The vessels were harvested 14 days later and fixed, and histology was determined as described. These investigations conform with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (publication No. 85-23, revised 1996) and Temple University.

Immunohistochemistry, Morphometry, and Statistics

Immunohistochemistry was performed as described previously with ADAM17 antibody (Abcam 39163), proliferating cell nuclear antigen (PCNA) antibody (Millipore P12004), and phospho-TyrEGFR (Cell Signaling 2236). For the quantification of PCNA and phospho-TyrEGFR, the percentages of PCNA-positive and phospho-TyrEGFR-positive nuclei surrounded by pEGFR-positive staining were counted, respectively, in the neointima as described previously. For vascular morphometry, digitized images were averaged from ≥3 representative stained tissue sections using Image Pro Plus (Media

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From the Cardiovascular Research Center and Department of Physiology, Temple University School of Medicine, Philadelphia, PA.
A.T. and K.K. contributed equally to this work.
Correspondence to Satoru Eguchi, Cardiovascular Research Center, Temple University School of Medicine, 3500 N Broad St, Philadelphia, PA 19140.
E-mail seguchi@temple.edu
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Cybernetics). The circumference of the lumen, the area encircled by the internal elastic lamina, and the external elastic lamina were quantified. The medial and intimal areas were then calculated. The data are presented as mean±SE. Groups were compared using ANOVA followed by Student t test. The null hypothesis was rejected when P<0.05.

**Results**

To examine the role of ADAM17 in participating vascular remodeling, expression of ADAM17 was assessed in the carotid artery after a balloon angioplasty. The presence of ADAM17-positive cells was observed in the neointima lesion compared with a control uninjured carotid artery, which has a weak staining in the medial layer (Figure 1). To study the involvement of ADAM17 in the neointima formation, wtADAM17 adenovirus or dnADAM17 adenovirus was delivered on arterial injury. wtADAM17 adenovirus enhanced, whereas dnADAM17 adenovirus reduced, the intima:media ratio compared with the GFP adenovirus (Figure 2). The efficiency of gene transfer was confirmed with immunohistochemical analysis of the samples with anti-ADAM17 antibody (Figure S1, available in the online Data Supplement at http://hyper.ahajournals.org).

To study the role of ADAM17 in regulating VSMC proliferation in response to arterial injury, PCNA-positive cells were evaluated in the above conditions. PCNA-positive cells were more abundant in the neointima with the wtADAM17 gene transfer and were less abundant with the dnADAM17 gene transfer compared with control GFP-delivered arteries (Figure 3). To assess EGFR activation in the neointima in response to arterial injury, phospho-EGFR staining was evaluated. Uninjured media was faintly stained with phospho-EGFR antibody. Compared with the GFP control, neointimal phospho-EGFR-positive cell numbers were enhanced with wtADAM17 and reduced with dnADAM17 (Figure 4).

![Figure 1. ADAM17 expression in response to arterial injury. Histological analysis of ADAM17 expression in arterial cross-sections obtained after balloon injury. Arterial sections obtained on day 14 after injury were stained with ADAM17 antibody or with control IgG (×200 magnification). Representative sections (each from n=3) are shown.](http://hyper.ahajournals.org/)

![Figure 2. ADAM17 is involved in neointima formation in response to arterial injury. The effect of ADAM17 adenovirus on arterial neointima formation after balloon injury was analyzed. Representative sections (×40 magnification) are shown. Fourteen days after injury, the common carotid artery was stained, and the area of neointima and media were quantified. Data are mean±SE of sections from 4 to 6 rats. *P<0.05 vs the GFP adenovirus-infected control.](http://hyper.ahajournals.org/)

![Figure 3. Histological analysis of cell proliferation in arterial cross-sections obtained after balloon injury. Arterial sections obtained on day 14 after injury with infection of adenovirus encoding GFP, wtADAM17, or dnADAM17 were stained with the antibody for PCNA. Representative sections (each from 3 rats, ×200 magnification) are shown. The graph shows quantitative analysis of PCNA-positive cells in the neointima from the 3 high-powered fields (mean±SE). *P<0.05 vs the GFP adenovirus-infected control.](http://hyper.ahajournals.org/)
Discussion

Although reduced ADAM17 mRNA expression in the liver of atherosclerosis-resistant mice has been reported recently, our data demonstrate a critical role of ADAM17 for neointimal hyperplasia in response to an arterial injury. In line with our observation of the enhanced ADAM17 expression in neointima, strong ADAM17 expression has been detected in intimal lesions of apolipoprotein E/H/H/H mice and human atherosclerotic plaque. ADAM17 expression was also higher in patients with acute myocardial infarction than those with stable angina pectoris. Moreover, single-nucleotide polymorphisms of ADAM17 are associated with increased serum tissue necrosis factor- and the risk of cardiovascular death in patients with coronary artery disease. Therefore, enhanced ADAM17 expression/activity could be a novel predictor of ongoing lesion formation in the vasculature.

EGFR activation has long been implicated in experimental models of restenosis; however, the mechanism through which this occurs in vivo is ill defined. In this regard, there have been many mechanisms proposed to mediate EGFR activation associated with vascular remodeling, including intracellular mechanisms without the participation of any EGFR ligand (of which the precursor needs to be processed by a metalloprotease). As such, we believe our nonpharmacological data supporting a critical role for ADAM17 in EGFR activation leading to neointimal hyperplasia will move the field forward.

At present, the identity of the EGFR ligand(s) shed by ADAM17 responsible for the in vivo EGFR activation remains unknown. ADAM17 is a major convertase of certain EGFR ligands, including heparin-binding EGF-like growth factor, transforming growth factor-α, amphiregulin, and epiregulin in mouse embryonic cells. In cultured VSMCs, heparin-binding EGF-like growth factor has been reported to be responsible for EGFR transactivation and subsequent extracellular signal–regulated kinase activation induced by angiotensin II and other G protein–coupled receptor agonists. Moreover, it has been reported that low flow-induced vascular remodeling was prevented in heparin-binding EGF-like growth factor−/− mice. Epiregulin produced by ADAM17 could also be involved in the neointimal hyperplasia. It is required for the EGFR transactivation and proliferation of VSMCs stimulated by fractalkine (CX3CL1). Moreover, epiregulin is a potent VSMC-derived mitogen induced by angiotensin II or endothelin and is expressed in rat carotid artery after angioplasty and in human atherosclerotic arteries. Likewise, there is the potential for ADAM17-dependent production of transforming growth factor-α and/or amphiregulin in mediating vascular neointima formation, because they are both implicated in pathological vascular remodeling. Therefore, it is likely that ADAM17 mediates EGFR transactivation in response to arterial injury through multiple EGFR ligands rather than through one single ligand.

In addition to the EGFR ligand precursors mentioned above, ADAM17 participates in the ectodomain shedding of cell surface proteins of which processing will produce mature cytokines/chemokines and other bioactive factors or lead to inactivation or modulation of the receptors or adhesion molecules. Therefore, other than EGFR activation, other ADAM17-dependent shedding/modulation events may collaboratively contribute to the initiation and/or progression of the neointimal remodeling. For example, among the known ADAM17 substrates, the production of tissue necrosis factor-α, fractalkine/CX3CL1, stem cell factor/kit ligand, or macrophage colony-stimulating factor 1 and inactivation of p75 tissue necrosis factor receptor or p75 neurotrophin receptor appear to be relevant for pathological vascular remodeling. Also, the effects of ADAM17 on various cell adhesion molecules should be considered when trying to evaluate the mechanisms through which ADAM17 influences neointimal remodeling.

Limitations of the current study include the lack of identification of the responsible ADAM17 substrate(s), as men-
tioned above. Addressing this critical issue is expected to significantly advance knowledge about ADAM involvement within the cardiovascular system. Although ADAM substrate identification and involvement have been assessed in vitro experiments (biochemical assays with the recombinant protease and candidate substrates, reporter-based shedding assays in cultured cells, flow cytometer to detect loss-of-cell surface precursor, or culture medium detection of the cleaved products), the list of ADAM17 substrates continues to grow. Indeed, recently developed “degradomics” approaches are anticipated to expand the list of potential substrates even further. In combination, the sheer number of ADAM17 are anticipated to expand the list of potential substrates even further. ADAM17 plays an important role in neointima formation and pathophysiology. Further expansion of research is, therefore, expected to determine global and tissue-specific roles of ADAM17 activity in regulating cardiovascular physiology and pathophysiology.

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### Disclosures

None.

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Cardiovascular Research Center and Department of Physiology, Temple University School of Medicine, Philadelphia, PA.

Online figure S1 and the figure legend
Figure S1. Histological analysis of ADAM17 expression in arterial cross-sections obtained after balloon injury. Arterial sections obtained on day 14 after injury with infection of adenovirus encoding GFP or dnADAM17 or from uninjured artery were stained with the antibody for ADAM17. Representative sections (each from 3 rats, x200 magnification) are shown. Note that adenoviral gene transfer of dnADAM17 markedly enhanced the ADAM17 positive area at the neointima even though the neointima formation was much less than the control GFP adenovirus-treated artery. Therefore, the strong signal reflects the efficiency of the ADAM17 gene transfer in the vasculature.