Local Tenomodulin Absence, Angiogenesis, and Matrix Metalloproteinase Activation Are Associated With the Rupture of the Chordae Tendineae Cordis

Naritaka Kimura, MD; Chisa Shukunami, DDS, PhD; Daihiko Hakuno, MD, PhD; Masatoyo Yoshioka, MD, PhD; Shigenori Miura, PhD; Denitsa Docheva, PhD; Tokuhiro Kimura, MD; Yasunori Okada, MD, PhD; Ryohei Yozu, MD, PhD; Junjiro Kobayashi, MD, PhD; Hatsue Ishibashi-Ueda, MD, PhD; Yuji Hiraki, PhD; Keiichi Fukuda, MD, PhD

Background—Rupture of the chordae tendineae cordis (CTC) is a well-known cause of mitral regurgitation. Despite its importance, the mechanisms by which the CTC is protected and the cause of its rupture remain unknown. CTC is an avascular tissue. We investigated the molecular mechanisms underlying the avascularity of CTC and the correlation between avascularity and CTC rupture.

Methods and Results—We found that tenomodulin, which is a recently isolated antiangiogenic factor, was expressed abundantly in the elastin-rich subendothelial outer layer of normal rodent, porcine, canine, and human CTC. Conditioned medium from cultured CTC interstitial cells strongly inhibited tube formation and mobilization of endothelial cells; these effects were partially inhibited by small-interfering RNA against tenomodulin. The immunohistochemical analysis was performed on 12 normal and 16 ruptured CTC obtained from the autopsy or surgical specimen. Interestingly, tenomodulin was locally absent in the ruptured areas of CTC, where abnormal vessel formation, strong expression of vascular endothelial growth factor-A and matrix metalloproteinases, and infiltration of inflammatory cells were observed, but not in the normal or nonruptured area. In anesthetized open-chest dogs, the tenomodulin layer of tricuspid CTC was surgically filed, and immunohistological analysis was performed after several months. This intervention gradually caused angiogenesis and expression of vascular endothelial growth factor-A and matrix metalloproteinase in the core collagen layer in a time-dependent manner.

Conclusions—These findings provide evidence that tenomodulin is expressed universally in normal CTC in a concentric pattern and that local absence of tenomodulin, angiogenesis, and matrix metalloproteinase activation are associated with CTC rupture. (Circulation. 2008;118:1737-1747.)

Key Words: angiogenesis • chordae tendineae cordis • metalloproteinases • tenomodulin • valves

Although the heart is a vascularized organ, the cardiac valves and chordae tendineae cordis (CTC) are avascular tissues.1 This avascularity is abrogated in several valvular heart diseases (VHDs).2–4 Chondromodulin-I, which is an antiangiogenic factor isolated from bovine cartilage,5–8 is also expressed in the eye and is critically involved in the maintenance. Recently, we have reported that chondromodulin-I was abundantly expressed by the valvular interstitial cells in normal cardiac valves.9 Gene targeting of chondromodulin-I resulted in enhanced vascular endothelial growth factor (VEGF)-A expression, lipid deposition, and calcification in the cardiac valves of aged mice. In human VHDs, including infective endocarditis, rheumatic heart disease, and atherosclerosis, VEGF-A expression, neovascularization, and calcification were observed in areas of chondromodulin-I downregulation. These findings provide evidence that chondromodulin-I plays a pivotal role in maintaining normal valvular function by preventing angiogenesis that might lead to VHD.
Rupture of the CTC is a well-known cause of mitral regurgitation. To elucidate the molecular mechanism, we investigated the expression of chondromodulin-I in the CTC. Unexpectedly, both normal and ruptured CTC lacked expression of chondromodulin-I, suggesting that the mechanism underlying the avascularity or protective function of the CTC differed from that operating in cardiac valves, even though the cardiac valves and CTC lie in proximity to each other and have a similar avascular appearance. The atrioventricular valve leaflets and CTC comprise diverse cell lineages and highly organized matrices that are populated by cartilage10,11 and tendon12,13 cell types, respectively. The cartilage cell markers aggrecan and Sox9 are observed in valvular leaflets during embryogenic valvulogenes, whereas the tendon-associated genes Scleraxis and Tenascin are expressed in the CTC.14,15 On the basis of these observations and our previous findings regarding chondromodulin-I in cardiac valves,9 we speculated that CTC avascularity might be related to the avascular properties of tendon rather than those of cartilage.

Recently, we isolated tenomodulin, which is a novel chondromodulin-I–related gene with 33% amino acid identity. Tenomodulin is a 317–amino acid glycoprotein found in hypovascular tissues, such as tendons, ligaments, the epimysium, and eyes.16–19 Tenomodulin contains BRICHOS and cysteine-rich domains20 and has antiangiogenic activities. It is processed in vivo in certain tissues, and the proteolytically cleaved, 16-kDa C-terminal domain promotes tenocyte proliferation.21,22 In the present study, we investigated whether tenomodulin is expressed in the CTC and its potential involvement in CTC avascularity. We also investigated the cause of the rupture of CTC by comparing the normal and ruptured CTC from the viewpoint of angiogenesis and the involvement of tenomodulin.

Methods

Animals

Wild-type ICR mice were purchased from Japan CLEA (Tokyo, Japan). Japanese White rabbits were purchased from Sankyo Laboratory Service Corporation (Tokyo, Japan). Porcine eyes and hearts were obtained from NARC Corporation (Chiba, Japan). All experimental procedures and protocols were approved by the animal care and use committees of Keio University and were in accordance with the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Reverse Transcription Polymerase Chain Reaction

Total RNA was isolated with the use of Trizol reagent (GIBCO-BRL) and treated with DNase I (Roche). Reverse transcription polymerase chain reaction (RT-PCR) was performed as described previously with the following primers: murine tenomodulin, 5′-AGAATGGCAATGTTGTTGTC-3′ (forward), 3′-CTGACCTCCTTGGTACCAG-5′ (reverse); murine GAPDH, 5′-TTCACGGCA-CAGTCAAGG-3′ (forward), 3′-CTGAGTCTGGTCATGAG-5′ (reverse); porcine tenomodulin, 5′-GGTGTTCCCTTCAAGTGAAAG-3′ (forward), 3′-CTGCTTCTGTAGTACCAG-5′ (reverse); porcine GAPDH, 5′-TGATGACATCAAGAAGTGGTGAAG-3′ (forward), 3′-TCCTTGGAGGCATCTTGGCAT-5′ (reverse).

Immunohistochemical and Immunofluorescence Staining

Conceivably, both normal and ruptured CTC were perfused from the apex with phosphate-buffered saline, perfusion-fixed with 4% paraformaldehyde and incubated overnight at 4°C and 4% paraformaldehyde, and then embedded in paraffin. Before application of the primary antibodies, paraffin was removed from the sections in xylene, and the sections were heated in a microwave oven in 10 mmol/L citrate buffer solution (pH 6.0) (Muto Pure Chemicals Co, Japan) for 3 minutes. After sections were rinsed in phosphate-buffered saline, they were incubated with ImmunoBlock (Dainippon Sumitomo Pharma, Osaka, Japan) 1 hour in room air and incubated overnight at 4°C with 5% normal rabbit serum and rabbit polyclonal antibody to tenomodulin,17,18 rabbit polyclonal antibody to VEGF-A (1:200 dilution; Santa Cruz Biotechnology, Santa Cruz, Calif), von Willebrand factor (vWF) (1:200 dilution; Laboratory Vision Corporation), elastin (1:50 dilution; Elastin Products Co), collagen type I (1:50 dilution; Rockland), matrix metalloproteinase (MMP)-1 (Daichi Fine Chemical),23 MMP-2 (Daichi Fine Chemical),23 MMP-3 (Daichi Fine Chemical),23 MMP-9 (Daichi Fine Chemical).23 MMP-13 (1:100 dilution; Biogenes),24 CD11b (1:200 dilution; BD Pharmingen), CD14 (1:50 dilution; Santa Cruz Biotechnology), and vimentin (1:20 dilution; Sigma-Aldrich, St Louis, Mo). Immunohistochemical signals were detected by applying 0.05% 3,3′-diaminobenzidine tetrahydrochloride (Sigma-Aldrich) containing 0.01% hydrogen peroxide in 0.05 mol/L Tris-buffered saline (pH 7.6) as a chromogenic substrate. The sections were then counterstained with hematoxylin, dehydrated in a graded ethanol series, and mounted in Permount (Fisher Scientific).

For immunofluorescence studies, the sections were incubated with secondary antibodies conjugated with Alexa 488 or Alexa 546 (Molecular Probes, Carlsbad, Calif). Slides were observed under a confocal laser-scanning microscope (LSM 510 META; Carl Zeiss, Chester, Va). Optical sections were obtained at 1024×1024 pixel resolution and analyzed with the use of LSM software (Carl Zeiss). We substituted nonimmune rabbit serum for primary antibodies as a negative control for each immunostaining experiment.

Quantitative analysis of the stained area was performed by converting images to monochrome with optimum saturation and counting the black pixels with the use of NIH Image software.

Isolation of Adult Rabbit CTC Interstitial Cells

The hearts were dissected from anesthetized 12-week-old Japanese White rabbits. Primary culture of the CTC was examined by modifying the protocol for cardiac valvular interstitial cells26 and Achilles tendons.27 Briefly, the CTC was rapidly removed, and the superficial endothelial cells were removed by cotton swab, chopped under a stereomicroscope, and used for the explant culture. Pieces that measured 1×1 mm were cut from the tissue, placed in 12-well collagen-coated dishes (Iwaki), and grown in Dulbecco’s modified Eagle’s medium (Sigma-Aldrich) with 50% fetal bovine serum (FBS), additional 1 mL Dulbecco’s modified Eagle’s medium with 50% fetal bovine serum was added and left for another 24 hours at 37°C. After the medium and tissue pieces were removed, Dulbecco’s modified Eagle’s medium with 10% fetal bovine serum was added, and the cells were cultivated at 37°C. Medium was changed every 3 days. Conditioned medium was obtained from confluent CTC interstitial cells 3 days after the medium was changed and used in further analyses.

Cell Culture

Human coronary artery endothelial cells (HCAECs) were purchased from Takara Biotechnology, maintained according to the manufacturer’s instructions, and used at passages 3 to 5 in the present study.

Human Samples

Samples comprising 16 CTC were collected from 15 patients undergoing mitral valve replacement or plasty due to its rupture and...
Results

Expression of Tenomodulin in Normal CTC

Initially, we investigated whether tenomodulin was expressed in normal cardiac valves and CTC. Tenomodulin transcripts were first detected in the murine heart at embryonic day 14.5 and were expressed continuously in adulthood (Figure 1A). It was expressed specifically in the CTC but not in the atrium, ventricle, or cardiac valves (Figure 1B). Western blotting with antibodies specific for the C-terminal portion of tenomodulin identified the 45-kDa glycosylated and 40-kDa nonglycosylated forms of tenomodulin in porcine CTC; these proteins were also detected in the eye (Figure 1C). Interestingly, the CTC, but not the eye, was immunopositive for the 16-kDa C-terminal cleaved domain of tenomodulin, suggesting truncation of the C-terminus to produce the secreted form. Western blot analysis for the N-terminal domain of tenomodulin identified the 45-kDa glycosylated and 40-kDa nonglycosylated forms of tenomodulin. Eye was used as positive control. Note that the CTC expressed not only 45- and 40-kDa bands but also a 16-kDa band, although the eye expressed only the 45- and 40-kDa bands. This suggests that the C-terminal domain is processed in the CTC but not in the eye.

Figure 1. Expression of tenomodulin in CTC. A, Temporal expression of tenomodulin (TEM) in the mouse heart. RT-PCR was performed on samples from embryonic and adult hearts. Positive signals were seen in the embryonic heart from embryonic day (E) 14.5 onward. Adult mouse eyes were used as positive controls. B, RT-PCR of tenomodulin in the porcine heart. Tenomodulin mRNA was specifically expressed in the CTC but not in the atrium, ventricle, or cardiac valves. C, Western blot analysis for tenomodulin in the porcine cardiac valve and CTC with the use of antibody specific for the C-terminus (top panel) and N-terminus (middle panel) of tenomodulin. Eye was used as positive control. Note that the CTC expressed not only 45- and 40-kDa bands but also a 16-kDa band, although the eye expressed only the 45- and 40-kDa bands. This suggests that the C-terminal domain is processed in the CTC but not in the eye.

Tenomodulin Secreted From CTC Interstitial Cells Has Antiangiogenic Activity

We investigated whether CTC interstitial cells produce tenomodulin and the effect that this might have on the tube elastica van Gieson staining (Figure 2B), and immunohistochemistry revealed 3 layers in the normal human CTC, ie, the superficial endothelial layer (Figure 2E), the elastin-rich mid layer (Figure 2G), and the collagen type I–rich core layer (Figure 2H). This structure was consistent with that reported previously.1 Tenomodulin was restricted to the elastin-rich mid layer (Figure 2C) and was not detected in the other layers. The normal CTC showed no expression of chondromodulin-I (Figure 2D) or VEGF-A (Figure 2F), and there was no abnormal vessel formation. Tenomodulin was deposited at the interstitial space of the elastin-rich layer, although it did not colocalize directly with elastin (Figure 2I).
CTC interstitial cells lost their migratory capacity compared to the control condition (Figure 4B). These results imply a pivotal role for tenomodulin as an angiogenesis inhibitor in the CTC.

Marked Downregulation of Tenomodulin and Abnormal Vessel Formation in the Ruptured CTC

We examined specimens from 16 patients with CTC rupture (Figure 5A). The ruptured areas contained numerous large abnormal vessels in the mid layers and core layers, and there was marked tissue degeneration. Elastin was preserved, whereas tenomodulin was markedly downregulated in the ruptured area but not in remote nonruptured areas. These tenomodulin-poor areas expressed VEGF-A and contained the aforementioned large abnormal vessels. In addition, their inner surfaces were coated with vWF-positive endothelial cells rather than smooth muscle cells. Computer image analysis showed that the total cell numbers, vWF-positive cell numbers, and the percentages of VEGF-A–positive areas were markedly increased, whereas the proportions of tenomodulin-positive areas in the ruptured CTC were markedly decreased (Figure 5B to 5E). The adjacent nonruptured CTC obtained at autopsy and during mitral valve replacement revealed normal structures without abnormal vessel formation or aberrant MMP or VEGF-A expression (data not shown). These findings indicated that the CTC ruptures in a fragile area where numerous abnormal vessels are created and tenomodulin expression was locally downregulated.

Expression of MMPs and Cell Infiltration in the Ruptured CTC

The ruptured area of the CTC showed strong expression of MMP-1 and MMP-2 and moderate expression of MMP-13, which corresponded to the expression of VEGF-A (Figure 6A). In contrast, MMP-3 expression was weak, and MMP-9 was not detected. No MMP signals were detected in the normal CTC or in the nonruptured area. High numbers of inflammatory cells positive for CD11b, CD14, and vimentin infiltrated the ruptured area but not the normal CTC or the nonruptured area. The quantitative analyses are shown in Figure 6B to 6E. These findings suggest that abnormal vessel formation in the CTC is accompanied by MMP activation and infiltration of inflammatory cells.

Mechanical Stretching and Hypoxia Suppress the Expression of Tenomodulin by CTC Interstitial Cells

To investigate the cause of tenomodulin downregulation, we investigated whether tenomodulin expression by CTC interstitial cells was affected by various stimuli, such as mechanical stretching, hypoxia, or oxidative stress. CTC interstitial cells would be expected to experience these types of stimuli.
Figure 3. Expression of tenomodulin in CTC interstitial cells and its effect on tube formation in HCAECs. A, Rabbit CTC interstitial cells (CIC) after postexplant culture. At 7 days, CTC interstitial cells showed a cobblestone-like or spindle-like appearance. At 14 days, CTC interstitial cells exhibited a fibroblast-like appearance. Ex indicates explant of CTC. CTC interstitial cells were negative for acetyl-LDL-DiI staining; HCAECs are shown as a positive control (inset). Immunofluorescence staining of tenomodulin (TEM) in rabbit CTC interstitial cells and NIH3T3 cells is shown; nuclei were stained with ToPro-3. B, Effect of siRNA on tenomodulin expression in cultured CTC interstitial cells. RT-PCR for tenomodulin was performed. Lane 1, CIC; lane 2, CIC+siRNA specific to tenomodulin; lane 3, CIC+control siRNA; lane 4, eye for positive control of tenomodulin. C, Tube formation assay. CIC-CM inhibited tube formation of endothelial cells on Matrigel. Representative micrographs of tube formation of HCAECs are shown. Tube formation was significantly suppressed by CIC-CM but not by NIH3T3-CM (negative control). Treatment of CTC interstitial cells with siRNA specific to tenomodulin but not control siRNA reduced the CIC-CM–induced suppression. Recombinant C-terminal tenomodulin represented the conditioned medium of NIH3T3 cells transfected with C-terminal tenomodulin expression plasmids. D, Quantitative analysis of tube lengths in tube formation assay. *P<0.01 vs control.
when subjected to abnormal forces (as in hypertension) or during inflammation (as in infective endocarditis). Initially, we stimulated the CTC interstitial cells using a cell-stretching device, and 6 hours later, the expression of tenomodulin was not detectable (Figure IIA in the online-only Data Supplement). Next, CTC interstitial cells were incubated under the hypoxic condition of almost no oxygen. Although the cells were alive, the expression of tenomodulin was not detected as early as 1 hour later (Figure IIB in the online-only Data Supplement). Finally, CTC interstitial cells were treated with several concentrations of antimycin A, which mediates oxidative stress. Tenomodulin expression was observed at an antimycin A concentration \( \leq 10 \mu g/mL \) but was undetectable at \( 30 \mu g/mL \) antimycin A (Figure IIC in the online-only Data Supplement). These findings suggest that various stimuli, such as mechanical stretching, hypoxia, and oxidative stress, cause the down regulation of tenomodulin expression in CTC.

Absence of the Tenomodulin Layer Induces Angiogenesis and MMP Activation

We investigated the effects of the tenomodulin layer on angiogenesis and MMP activation in the collagen-rich core layer. The CTC of the tricuspid valve was examined in an anesthetized canine model, and the tenomodulin-rich layer was removed by filing (Figure 7A). Hemodynamic measurement revealed that there were no significant differences in heart rate, systolic pressure, diastolic pressure, pulmonary arterial pressure, pulmonary capillary wedge pressure, right ventricular systolic pressure, right atrial pressure, and cardiac output between the control (presurgery) dogs and the dogs at 3 months after surgery. Color Doppler echocardiography revealed no significant increases in tricuspid regurgitation at 3 months after surgery.

HE staining and immunostaining for vWF revealed abnormal vessel formation in the core layer next to the surgically filed tenomodulin-deficient area at 3 months (Figure 7B, 7C). Immunohistochemical analyses were performed at 1 and 3 months, at which point the acute inflammation had terminated. VEGF-A, MMP-1, MMP-2, and MMP-13 were observed from 1 month, with their expression extending to \( \approx \) 15% of the core layer depth (Figure 7D). At 3 months, the expression of these factors was stronger and extended to \( \approx \) 37% of the core layer depth. Moreover, abnormal neovascularization was observed only at 3 months, indicating that angiogenesis and MMP activation are caused by loss of the tenomodulin-rich layer rather than operation-induced inflammation (Figure 7E to 7G).
Discussion

Despite their clinical importance, little is known about the mechanisms underlying VHDs. The present study reveals a key role for tenomodulin as a potent antiangiogenic factor in the prevention of atrioventricular valvular regurgitation after CTC rupture. We show for the first time that both the C-terminal and N-terminal domains of tenomodulin are expressed persistently in the mid layer of normal CTC, whereas these proteins are downregulated in the diseased CTC. Moreover, the cleaved C-terminal domain of tenomodulin secreted from CTC interstitial cells is a critical antagonist of angiogenesis. Because cardiac valves are flow-regulating tissues in a dynamic chambered pump, the CTC are subjected to mechanical stress and damage to the endothelial cell lining of the outer layer. Tenomodulin probably protects the CTC from the inflammation and vascularization that result from mechanical damage. To confirm this hypothesis, we analyzed the tenomodulin expression profiles of the CTC in the normal and diseased states.

The observed expression patterns and biological activities suggest that tenomodulin protects against both angiogenesis and degeneration of the core layer, analogous to the surface coating applied to steel to prevent internal corrosion. Indeed, observations of the ruptured CTC revealed that VEGF-A and MMPs were strongly activated with increased formation of abnormal vessels, resulting in degeneration of the CTC. More importantly, these areas of degeneration corresponded to regions of local absence of tenomodulin. The dog experiments clearly support our hypothesis that angiogenesis and degeneration occur progressively in areas from which the tenomodulin layer has been removed. These findings suggest that CTC rupture is not accidental but can occur when and where the CTC is weakened by angiogenesis, MMP activation, and the infiltration of inflammatory cells secondary to the loss of or damage to the tenomodulin layer. The molecular mechanism through which a local deficit of tenomodulin causes VEGF-A expression remains unknown. We assume that
Figure 6. A, Immunohistochemistry of CTC from human autopsies and surgical samples with the use of anti-MMP-1, -MMP-2, -MMP-3, -MMP-9, -MMP-13, -CD11b, -CD14, and -vimentin antibodies. Normal represented the samples from autopsies with normal CTC. The chordae rupture represented the ruptured area of the CTC from surgical samples. B, Quantitative analysis of the percentage of MMP-1–immunostained area in the normal autopsied sample, the nonruptured area (NRA), and ruptured area (RA) of surgical CTC specimen. C, Quantitative analysis of the percentage of MMP-2–immunostained area. D, Quantitative analysis of the percentage of MMP-13–immunostained area. E, Quantitative analysis of the number of CD14$^+$ cells in CTC. *$P<0.01$ vs control.
either the downregulation of tenomodulin in CTC interstitial cells or the loss of tenomodulin-producing cells owing to long-standing mechanical stress or inflammation leads to the infiltration of endothelial cells and inflammatory cells, resulting in the activation of MMPs and the expression of VEGF-A. Tenomodulin plays a major role as an antiangiogenic factor in CTC; however, our present data do not exclude the possibility of the existence of other antiangiogenic factors. This should be clarified in the future. We propose that tenomodulin loss occurs after (1) acute inflammation, as in infective endocarditis and rheumatic fever, and (2) excessive mechanical stress to valves, as in trauma, hypertension, and mitral valve prolapse.

The Achilles tendon is one of the most common sites of rupture. It ruptures spontaneously in the majority of patients, without substantial trauma. Local vascularization is implicated in the etiology of this rupture; high levels of VEGF-A are expressed by the tenocytes of the Achilles tendon.
ruptured Achilles tendons but not by the tenocytes of normal adult Achilles tendons. 35,36 Hypercellularity, marked vessel formation, and increased MMP expression lead to weakening of the normal tendon structure and subsequent decrease in the mechanical strain tolerance, leading to spontaneous rupture.36,37 The similarities between these earlier findings and those of the present study suggest that locally augmented mechanical stress or inflammation causes local defects in tenomodulin and induces VEGF-A expression, which may lead to MMP activation, angiogenesis, and degeneration of the CTC to the extent that it ruptures. The present findings support the notion of preclinical investigations of tenomodulin and proteins of similar function as therapeutic agents for the prevention of VHD due to rupture or elongation of the CTC. Understanding these mechanisms should form the basis for new therapeutic regimens for the treatment of VHD.

Sources of Funding
This study was supported in part by research grants from the Ministry of Education, Science, and Culture, Japan, and by the Program for Promotion of Fundamental Studies in Health Science of the National Institute of Biomedical Innovation, Japan.

Disclosures
None.

References
Valvular heart disease is a life-threatening disease. Although the overall incidence of rheumatic valvular heart disease has been decreasing continuously in developed countries, it has increased with respect to patient age. Rupture of the chordae tendineae cordis (CTC) is a well-known cause of mitral regurgitation, although its etiology remains unknown and surgical procedures have focused exclusively on treatment. Cardiac valves and the CTC are avascular tissues, and we have recently reported that cardiac valves express chondromodulin-I, which is an angioinhibitory factor purified from cartilage that plays a pivotal role in the maintenance of normal valvular function by preventing angiogenesis. In the present study, we show that tenomodulin, which is a chondromodulin-I–related antiangiogenic factor isolated from tendons, is concentrically expressed in normal CTC. Conditioned medium from cultured CTC interstitial cells showed a strong angioinhibitory effect, and the immunohistochemical analysis of human surgical samples showed that tenomodulin was locally absent in the ruptured areas of the CTC, in which abnormal vessel formation, strong expression of vascular endothelial growth factor-A and matrix metalloproteinases, and infiltration of inflammatory cells were observed, whereas these features were not observed in the normal or nonruptured areas. The tenomodulin layers of the tricuspid CTC of dogs were surgically filed, the animals were euthanized after several months, and immunohistological analyses were performed. Angiogenesis and the expression of vascular endothelial growth factor-A and matrix metalloproteinases in the core layer were observed in a time-dependent manner. The present findings support tenomodulin and unknown proteins of similar function as therapeutic agents for the prevention of CTC rupture. Understanding these mechanisms should form the basis for new therapeutic regimens for the treatment of valvular heart disease.
Local Tenomodulin Absence, Angiogenesis, and Matrix Metalloproteinase Activation Are Associated With the Rupture of the Chordae Tendineae Cordis


Circulation. 2008;118:1737-1747; originally published online October 6, 2008; doi: 10.1161/CIRCULATIONAHA.108.780031

Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2008 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circ.ahajournals.org/content/118/17/1737

Data Supplement (unedited) at:
http://circ.ahajournals.org/content/suppl/2008/11/06/CIRCULATIONAHA.108.780031.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation is online at:
http://circ.ahajournals.org//subscriptions/