Cardiac hypertrophy is initially an adaptive response to preserve cardiac function in response to several forms of stress. However, after sustained external load, hearts can evolve to a state of decompensated hypertrophy resulting in cardiac dilation and loss of contractile function. Whereas it is known that overload-induced cardiac hypertrophy involves the participation of angiotensin II, endothelin-1, and fibroblast growth factor-2, the molecular mechanisms responsible for the transition from compensated to decompensated hypertrophy are poorly defined.

Myocardial ischemia and diminished myocardial blood flow are predictors of poor prognosis in heart failure. Pressure or volume overload–induced cardiac hypertrophy is associated with a reduction in capillary density in a number of animal models. In addition, a reduced capillary bed has been described for the left ventricular hypertrophy that occurs in the intact parts of the heart with myocardial infarction. Recently, we have shown that a reduction in cardiac capillary density promotes contractile dysfunction in a transgenic mouse model where a constitutively active form of Akt1 is expressed from a cardiac-specific promoter. These results suggest that impaired vasculature could contribute to the transition from compensated to decompensated cardiac hypertrophy. However, this hypothesis has not been directly tested in a model of pathological hypertrophy, such as that induced by pressure overload of the heart.

Vascular endothelial growth factor (VEGF) is an endothelial cell mitogen that has an essential role in both vasculo- genesis and angiogenesis. VEGF regulates multiple angiogenic cellular responses, including survival, migration, and differentiation, through activation of Akt signaling within endothelial cells. VEGF is secreted from cardiomyocytes in response to extracellular stimuli. Mice engineered to express only a single spliced isoform of VEGF-A gene (VEGF<sub>120</sub>) or mice with cardiac-specific deletion of VEGF-A exhibit reduced capillary density and impaired contractility. These reports led us to hypothesize that VEGF may be required to maintain the capillary bed under conditions of cardiac stress.

It has been shown previously that intravenous administration of an adenoviral vector encoding the ligand-binding domain of VEGF receptor 2 (Flk1) fused to murine IgG2a Fc leads to systemic VEGF secretion and inhibition of angiogenesis in both tumor and ischemic hindlimb models of vessel growth. Here, we used the adenoviral vector encoding the ectodomain of Flk1 in a murine model of pressure overload hypertrophy. This treatment resulted in reduced myocardial capillary density, accelerated contractile dysfunction, and pathological cardiac remodeling. These findings indicate that VEGF-dependent capillarization is essential for compensatory cardiac hypertrophy in response to pressure overload.

Key Words: heart failure • remodeling • endothelial growth factors • hypertrophy
Methods

Animals
Study protocols were approved by the Institutional Animal Care and Use Committee at Boston University. Ten-week-old male C57BL/6 mice were used in this study. Transverse aortic constriction (TAC) was performed as described previously in detail.22 Sham-treated animals underwent open chest surgery but not transverse aortic constricting. After 2 weeks of surgery, mice were subjected to transthoracic echocardiography and cardiac catheterization to determine heart rate, proximal aortic pressure, and left ventricular end-diastolic (LVED) pressure. Animals were then euthanized, and the hearts were weighed and harvested for additional analysis.

Adenovirus-Mediated Gene Transfer
Adenovirus vectors encoding Flk1-Fc and control Fc fragment were described previously.19 We injected 2×10⁸ plaque-forming units of Ad-Flk1-Fc (Ad-Flk) or Ad-control Fc (ad-cont) into the jugular vein of mice 3 days before TAC.

Echocardiography
Transthoracic echocardiography was performed with an Acuson 256 sector scanner equipped with a 13-MHz broadband transducer. All of the recordings were performed with conscious animals.23

Quantitative Real-Time PCR
Total RNA was prepared by Qiagen using protocols provided by the manufacturer. cDNA was produced using ThermoScript RT-PCR Systems (Invitrogen). Real-time PCR was performed as described previously.24 Transcript levels of atrial natriuretic peptide (ANP), VEGF-A, and collagen III was determined as the relative number of transcripts to those of glyceraldehydes-3-phosphate dehydrogenase and normalized to the mean value of control hearts. Primers for ANP, VEGF-A, collagen III, and glyceraldehydes-3-phosphate dehydrogenase were as described.24–26

Histological Analysis
Heart sections were prepared as described27 and were stained with TRITC-conjugated BS-1 lectin to evaluate capillary density, fluores-

![Figure 1. Treatment with VEGF receptor decoy decreases the coronary vascular bed in hearts subjected to TAC. (A) TAC leads to an increase in VEGF-A transcript expression. (B) Representative images of TRITC-conjugated BS-1 lectin stained heart sections. Scale bars: 50 μm. (C) Quantitative analysis of capillary/myocyte ratio in Ad-cont- or Ad-Flk-treated mice at 2 weeks after sham operation or TAC. (D) Quantitative analysis of capillary density in Ad-cont- or Ad-Flk-treated mice 2 weeks after sham operation or TAC. Results are presented as mean±SEM (n=4 to 6 each). *P<0.05. #P<0.05 vs Ad-cont with sham.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Sham</th>
<th>TAC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>21.8±0.5</td>
<td>21.9±0.5</td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>99±3</td>
<td>101±4</td>
</tr>
<tr>
<td>Heart rate (bpm)</td>
<td>488±32</td>
<td>485±26</td>
</tr>
</tbody>
</table>

Results are presented as mean±SEM. *P<0.05 vs Ad-cont sham.
cein isothiocyanate-conjugated wheat germ agglutinin to evaluate myofiber size, and Masson’s trichrome for detection of myocardial interstitial fibrosis. To determine the capillary density, myofiber size, and myocardial interstitial fibrosis, we selected 10 fields randomly and calculated as described previously with the image analysis software NIH IMAGE.

Statistics Analysis
All of the data are presented as mean±SEM. Comparisons among groups were made by 1-way ANOVA, followed by Scheffe F test. A level of P<0.05 was accepted as statistically significant.

Results
VEGF Receptor Decoy Reduces Capillary Density in Hearts Subjected to TAC
Consistent with previous results, TAC led to a 2.9-fold (P<0.01) increase in VEGF-A transcript expression (Figure 1A). To investigate the links between coronary vasculature and compensatory cardiac hypertrophy, an adenoviral vector encoding a decoy VEGF receptor fused to the Fc fragment of IgG2a (Ad-Flk) or a control vector expressing only the Fc fragment (Ad-cont) was delivered to mice, and hearts were subjected to sham surgery or pressure overload resulting from TAC. Based on prior experiments, 2×10⁹ plaque-forming units of Ad-Flk or ad-cont were injected via the jugular vein. As shown in Table, there were no significant differences in body weight in each experimental group at 2 weeks after surgery or sham treatment. Mice exposed to TAC showed a significant increase in proximal aortic systolic blood pressure and heart rate compared with sham in both Ad-Flk and Ad-cont experimental groups.

Capillary status was evaluated by histology to determine the effect of Ad-Flk on the coronary vasculature (Figure 1B). In Ad-cont mice, TAC led to an increase in capillary/myocyte ratio at 2 weeks (Figure 1C), but the net myocardial capillary density decreased significantly (Figure 1D). Treatment with Ad-Flk blocked the increase in capillary/myocyte ratio and led to a further reduction in the capillary density in the myocardium relative to the Ad-cont group. In contrast, Ad-Flk had no effect on capillary/myocyte ratio or capillary density in sham-operated mice.

VEGF Receptor Decoy Reduces Impaired Cardiac Hypertrophy in Response to TAC
The heart weight/body weight ratio was significantly increased by TAC in control mice (Figure 2A). Treatment with
Ad-Flk led to a 54% decrease in TAC-induced cardiac growth. However, treatment with Ad-Flk had no effect on heart size in sham-treated animals. Analysis of myocyte cross-sectional area in histological sections corroborated these findings (Figure 2B). The calculated myocyte size increased in control mice that underwent TAC, but this increase was largely blocked in mice that were administered Ad-Flk before TAC (Figure 2C). Ad-Flk administration to sham mice had no effect on myocyte cross-sectional area. Echocardiographic measurements also showed that interven- tricular septum (data not shown) and posterior wall thickness (Figure 3A and 3B) were significantly increased by TAC in Ad-cont–treated mice, but these increases were suppressed by the administration of Ad-Flk before TAC.

**VEGF Receptor Decoy Accelerates Contractile Dysfunction and Pathological Cardiac Remodeling**

LVED dimension was significantly increased, and percentage of fractional shortening (%FS) was significantly decreased in Ad-cont–treated mice 2 weeks after TAC (Figure 3C and 3D). Treatment with Ad-Flk promoted the enlargement of LVED dimension and further decreased %FS in mice who underwent TAC (Figure 3E). In contrast, treatment with Ad-Flk had no effect on echocardiographic or hemodynamic parameters in sham-operated mice.

Myocardial interstitial fibrosis was evaluated to further investigate the consequence of diminished capillary density on cardiac remodeling (Figure 4A). There was a trend toward increased fibrosis after TAC in mice treated with Ad-cont, but this was not statistically significant (Figure 4B). However, treatment with Ad-Flk led to a large increase in fibrosis in the hearts subjected to TAC. Consistent with this observation, collagen III gene expression was significantly increased by Ad-Flk treatment in hearts subjected to TAC (Figure 4C). Finally, TAC led to an increase in the expression of the fatal-type cardiac gene ANP, and this upregulation was more pronounced in mice treated with Ad-Flk than Ad-cont (Figure 4D). Ad-Flk did not detectably affect fibrosis or collagen and ANP expression in sham-operated mice.

**Discussion**

In the present study, we demonstrated that sequestration of endogenous VEGF impairs adaptive cardiac hypertrophy in
response to pressure overload. We show that pressure overload leads to an upregulation of VEGF-A expression and an increase in the capillary/myocyte ratio. However, there is a net reduction in capillary density (capillaries/mm²), because the increase in capillarization (∼1.2-fold increase in capillaries/myocyte) does not keep pace with myocyte growth (∼2-fold increase in myocyte cross-sectional area). In animals treated with the ectodomain of Flk1, there was no increase in the capillary/myocyte ratio and, thus, a greater reduction in coronary capillary density. This corresponded to a rapid transition to heart failure as characterized by decreased %FS, increased LVED dimension and pressure, increased ANP and collagen III gene expression, and increased myocardial interstitial fibrosis. These findings suggest that VEGF is essential for coronary vascular network growth under conditions that promote compensatory cardiac hypertrophy and that a reduction in available VEGF contributes to the rapid progression from compensatory cardiac hypertrophy to failure. Notably, treatment with Flk-Fc had no effect on coronary capillary density or function in normal hearts of sham-treated animals. These data suggest that, in the absence of growth stimuli, normal contractile function can be maintained without the need for VEGF.

Previous studies have shown that VEGF is upregulated in myocardium in pathological conditions such as myocardial infarction, pressure overload, and hemodynamic overload. In vitro, it has been shown that VEGF is secreted from cardiomyocytes in response to various extracellular stimuli. Presumably, VEGF functions as a paracrine factor that is required to recruit new vessels as the heart grows, similar to what has been described for skeletal muscle hypertrophy. VEGF may also be required to preserve the myocardial capillary bed in pressure-overloaded hearts through its antiapoptotic actions on endothelial cells. Despite the upregulation in cardiac expression of VEGF under conditions of cardiac stress, "pathological" cardiac hypertrophy can be associated with a net loss of capillary density in some animal models. In contrast, cardiac growth associated with development, nutritional input, or vigorous exercise is associated with maintained or increased capillary density. Thus, the availability of VEGF may play a role in determining whether the phenotype of the growing heart is "physiological" or "pathological." Another finding of this study is that treatment with VEGF receptor decoy attenuated the extent of hypertrophy induced by pressure overload. Therefore, stress-induced heart growth may depend on the status of the vascular bed in a manner that is similar to tumor and fat pad growth. It has also been reported that knockout mice lacking cardiac VEGF exhibit a diminished capillary density and a small heart, suggesting that the endo-
Thelium is a source of growth factors for the heart during development. Similarly, liver or pancreas development requires factors released from newly formed endothelial cells for normal growth.38,39 Thus, the identification of paracrine factors released by coronary endothelial cells could lead to the identification of novel regulators or myocyte hypertrophy and function.40 In conclusion, our work shows that reductions in VEGF-dependent capillary density promote the rapid progression from compensatory cardiac hypertrophy to failure in pressure overloaded hearts. Thus, the status of the coronary vasculature can play a role in controlling heart function under conditions of cardiac stress. In this regard, patients with hypertrophic cardiomyopathy typically exhibit risk factors associated with endothelial cell dysfunction,41–43 and this combination could exacerbate the transition to heart failure. Finally, these data indicate that antiangiogenesis agents that target VEGF for the treatment of cancer could be detrimental for heart function in patients who also experience hypertrophic cardiomyopathy.

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References


23. Yang XP, Liu YH, Ruhale NE, Kunhara N, Kim HE, Carretero OA. Echocardiographic assessment of cardiac function in conscious and anes-


27. Fujio Y, Nguyen T, Weneker D, Kitsis RN, Walsh K. Akt promotes survival of cardiomyocytes in vitro and protects against ischemia-


Vascular Endothelial Growth Factor Blockade Promotes the Transition From Compensatory Cardiac Hypertrophy to Failure in Response to Pressure Overload

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