Post-ischemic myocardial fibrosis occurs independent of hemodynamic changes

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Abstract

Objectives: Myocardial fibrosis is a major component of ventricular remodeling after large myocardial infarction (MI). The present study tests the hypothesis that post-ischemic myocardial fibrosis can occur independent of hemodynamic changes. Methods: A mouse model of distal left coronary artery ligation was established to induce a small infarct (less than 15\% of the left ventricle) in order to avoid significant mechanical overload after permanent myocardial ischemia. Left heart catheterization was performed to evaluate the post-infarct hemodynamics. Tissues from both ischemic and non-ischemic myocardium were examined for mRNA and protein expression at 24, 72 h and 7 days after ligation.

Results: Heart/body weight ratio after ligation was increased by approximately 10\% over sham control although there is no statistically significant difference in hemodynamic parameters between the two groups. Non-ischemic myocardium distant from the infarct site showed molecular evidence of myocardial fibrosis 72 h and 7 days after ligation. There was marked up-regulation of mRNAs for extracellular matrix (ECM) proteins and their cross-linking enzyme, such as collagens type I, III and VI, and lysyl oxidase. Immunohistochemical study confirmed that the expression of these ECM proteins was significantly increased in the non-ischemic myocardium after 7 days. TGF-\textbeta1 was up-regulated after 72 h in both ischemic and non-ischemic myocardium. Conclusions: Molecular and histopathological findings demonstrate that abnormal myocardial fibrosis can be induced by a small infarct independent of secondary hemodynamic changes.

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1. Introduction

Large myocardial infarctions result in extensive left ventricle (LV) remodeling in both infarcted and non-infarcted zones [1,2]. Ventricular remodeling is a complex gross geometric alteration, which involves ultrastructural and microscopic changes of muscle fibers and their surrounding interstitial tissues. This results in the rearrangement of the components of the ventricular wall and subsequent development of chronic heart failure after ischemic myocardial injury [3–5]. Expansion of the infarcted area occurs early, whereas left ventricular chamber
dilatation and wall hypertrophy of the non-infarcted zone occur later [6]. Changes in the non-infarcted myocardium include hypertrophy of the cardiomyocytes, growth of the capillary network, and an increase in fibrillar collagens, types I and III. These microscopic alterations in the myocardial architecture in the non-ischemic myocardium are thought to be responsible for ventricular dysfunction in the chronic phase [5,7,8].

‘Ventricular remodeling’ refers to a chronic pathological condition of dysfunctional ventricular myocardium caused by a combination of several different factors such as ischemia, stretch due to pressure overload, stress secondary to myocardial scar formation and increased level of plasma levels of hormones and vasoactive peptides [9]. Mechanical stretch is believed to be an important inciting factor in inducing ventricular remodeling [2,9]. On the cellular level, three major microscopic alterations observed during the development of myocardial remodeling include myocyte hypertrophy, cell death and fibrosis [9]. Myocardial fibrosis is one of the major biological determinants in ventricular remodeling; it enhances myocardial stiffness, promotes the development of arrhythmias and hampers systolic ejection by rendering the myocardium heterogeneous in composition [10].

Based on results from animals subjected to a large myocardial infarction, it has been proposed that post-infarct ventricular remodeling can be attributed to both direct myocardial ischemic injury and secondary mechanical overload [2,9,16]. Post-ischemic ventricular remodeling has been exclusively studied in experimental animal models following creation of a large myocardial infarction by proximal ligation of the left anterior descending artery [11,13,14]. This results in ventricular enlargement, ventricular geometric alteration, secondary mechanical overload, and subsequently heart failure [15]. It has not been previously investigated, however, whether local myocardial ischemic injury itself without secondary hemodynamic changes can independently cause changes in myocardial architecture in the non-ischemic cardiac tissue.

In order to determine whether the ischemic injury by itself is sufficient for the ventricular remodeling in remote non-ischemic cardiac tissue after experimental coronary artery occlusion or whether the secondary mechanical alteration from a large infarction is primarily responsible for this effect, we have established a mouse model in which the left anterior descending artery is distally ligated. With this method, a smaller myocardial infarction (infarct size less than 15% of LV) can be induced and thus subsequent hemodynamic changes can be avoided. It has previously been demonstrated in a rat model that no significant impairment of left ventricular function occurs if the infarct size is limited to less than 30% of the left ventricular mass [17]. By utilizing this experimental model, we have demonstrated that primary local ischemic injury by itself can initiate the ventricular remodeling process in remote non-ischemic myocardium, despite the absence of secondary hemodynamic changes.

2. Methods

2.1. Animals

C57BL/6 male mice from age 12 to 18 weeks (weight 25–30 g) were purchased from the Jackson Laboratory. The investigation conforms with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (NIH Publication No. 85-23, revised 1998).

2.2. Experimental coronary artery ligation

Under general anesthesia with isoflurane (2%), the heart was exposed via a small left thoracotomy and suture ligation was placed at the distal 1/3 of the left anterior descending artery (LAD) with 6.0 silk suture. Upon ligation of distal LAD, the heart was immediately placed back to the intrathoracic space followed by closure of the skin suture and manual evacuation of pneumothoraces. Immediate post-operative mortality rate of this surgical procedure was 14.9% (17/114). Sham operation was performed in a same manner except that the LAD was left unligated. Once the mice survived the immediate post-operative period, there was complete survival until the date of subsequent examination (24, 72 h and 7 days after surgery).

2.3. Serum creatine kinase assay

After 24, 72 h or 7 days, the operated mice were again anesthetized with isoflurane inhalation, and their body weights were measured upon full anesthesia prior to the surgery. The heart was exposed via midline sternotomy and a blood sample was collected directly from the LV cavity for the measurement of serum creatine kinase (CK; U/dl) and total protein (g/dl). Serum CK activity was measured in a blind manner using a commercially available kit (Procedure 47-UV, Sigma, St. Louis, MO, USA).

2.4. Measurement of myocardial infarct size

After blood sampling for CK measurement, the heart was quickly excised and both atria and right ventricular free wall were removed. Left ventricular tissue was then frozen at −20 °C, and sliced into 1-mm thick sections perpendicular to the long axis of the heart using a heart slice chamber. Slices were incubated individually using a 24-well culture plate in 1% 2,3,5-triphenyltetrazolium chloride (TTC; Sigma) in phosphate-buffered saline at pH 7.4 at 37 °C for 5 min. In the presence of intact dehydrogenase enzyme systems (viable myocardium), TTC forms bright red formazan; infarcted areas lack dehydrogenase activity and therefore fail to stain. Left ventricular slices were photographed with a digital camera (1600×1200 dpi), and TTC staining positive areas (non-infarcted myocardium) and TTC staining negative areas (infarcted...
myocardium) were digitally measured using SigmaScan (SPSS, Chicago, IL, USA). The myocardial infarct size was expressed as a percentage of total left ventricular mass.

2.5. Left ventricular hemodynamic evaluation

After 7 days, both ischemic and sham operated mice were studied by invasive hemodynamic evaluation. The mice were anesthetized with intraperitoneal injections of 2.5% averton (0.02 ml/g, Sigma–Aldrich, St. Louis, MO, USA) 15 min prior to the experiments. After full anesthesia was obtained, the right carotid artery was exposed. A PE-10 catheter (Becton Dickinson, Mountain View, CA, USA) connected to a pressure transducer was inserted retrograde from the carotid artery to the left ventricular cavity to measure left ventricular pressure (LVP). The left ventricular systolic pressure (LVSP), left ventricular end diastolic pressure (LVEDP), first derivative of the LVP [(+dP/dt)max and (−dP/dt)max], and heart rate (HR) were obtained using computer algorithms and an interactive videographics program (Po-Ne-Mah Physiology Platform P3 Plus, Gould Instrument Systems, Valley View, OH, USA).

Dobutamine stress test was performed in a separate experiment 7 days after LAD ligation (five sham and six ischemic mice). After baseline hemodynamic measurements were obtained, dobutamine was administered (30 μg/kg/min, intravenous infusion) and the maximal HR and (+dP/dt)max increase to dobutamine stress were recorded.

2.6. Tissue preparation

In a separate series of studies, the hearts were excised from the thorax via median sternotomy under general anesthesia as described above at the end of the experimental procedure at 24, 72 h and 7 days after ligation. The weights of the hearts were measured after removal of the epicardial scar tissue and blood and washes with PBS. Hearts were then sectioned parallel to the LV long axis at the level of suture ligation (approximately the distal 1/3 of long axis). The distal part of the heart (ventricular apex) was collected as ischemic tissue (area-at-risk or AAR), and the myocardium approximately 2 mm above the suture site was collected as remote, non-ischemic tissue (area-not-at-risk or ANAR). The divided parts of myocardium were either kept in the liquid nitrogen for RNA isolation or embedded into tissue freezing media for histological study.

2.7. Semi-quantitative RT-PCR

RNA isolation was performed using guanidinium thiocyanate/cationic detergent solutions, according to the manufacturer’s protocol (Totally RNA™, Ambion, TX, USA). The resultant total RNA was treated with DNase I (Message Clean™, GeneHunter, TN, USA) to remove genomic DNA contamination. The quality of the RNA was assessed by running an aliquot of the RNA sample on a denaturing agarose gel. The first strand cDNA reaction was performed with MMLV reverse transcriptase (Life Technologies, MD, USA) and oligo-dT primers from 1 total RNA. PCR amplification was performed with the same amount of the first strand cDNA using Taq DNA polymerase (Qiagen). Preliminary experiments were carried out for each gene to select the optimal number of cycles to enable the amplification reaction to proceed in a linear range. PCR amplification of a constitutively expressed gene, GAPDH, was used as a control for the amount of input RNA. Three different series of RNA samples from each group were assayed. The sequences of the primers are shown in Table 1.

The PCR products were run on 1% agarose gels in the presence of ethidium bromide, and visualized by a FluorImager (Typhoon, Molecular Dynamics). Quantification of the images was performed with the Image-Pro Plus software (Media Cybernetics, MD, USA).

2.8. Immunohistochemistry

Non-ischemic myocardial tissue (ANAR; see Section 2.6) was embedded in tissue freezing medium (TBS™, Triangle Biomedical Sciences, NC, USA) for cryosection to study the time course of spatial protein localization after distal LAD ligation. A detailed protocol of immunohistochemistry has been described elsewhere [18]. Primary antibodies used were collagen type I (Rockland Immunochemicals, Gilbertsville, PA, USA; 1:100 dilution), collagen type III (Rockland Immunochemicals, 1:100 dilution), collagen type VI (a gift of Dr Rupert Timpl, Max

Table 1
Oligonucleotide primers used for RT-PCR analysis of gene expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Forward primer</th>
<th>Reverse primer</th>
<th>Product size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>α1(I) collagen</td>
<td>TGTGGACCTGCTGCTCTC</td>
<td>TTGTTCGCCCTGTCCACCCCT</td>
<td>462</td>
</tr>
<tr>
<td>α1(III) collagen</td>
<td>AGCTGGCATTCCTCAGACTT</td>
<td>GGTGTCACCTGACCTGTTT</td>
<td>431</td>
</tr>
<tr>
<td>α1(VI) collagen</td>
<td>CTGTGGTCGACACATTCAGG</td>
<td>ACGACTCTTCTTCTGTCGTC</td>
<td>581</td>
</tr>
<tr>
<td>Lysyl oxidase</td>
<td>ATATAGGGGCGGATGTCAGAG</td>
<td>CAATGTCACGCGCTACACAC</td>
<td>456</td>
</tr>
<tr>
<td>TGF-β1</td>
<td>CTGTTGACTAAGGCGCTGCC</td>
<td>TACAAGGTTGACGCCGACGAA</td>
<td>391</td>
</tr>
<tr>
<td>GAPDH</td>
<td>GATTGTTGCTGCGCATCAACGACC</td>
<td>TCCAGCAGACTCATCATCAG</td>
<td>236</td>
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</tbody>
</table>
Planck Institut für Biochemie, Martinsried, Germany; 1:1000 dilution) and PECAM-1 (CD31: BD PharMigen, San Diego, CA, USA; 1:100 dilution). Secondary antibodies used were Cy3 conjugated anti-rabbit antibody (1:1000 dilution) and Cy2 conjugated anti-rat antibody (1:800 dilution, both from Jackson ImmunoResearch Laboratories, West Grove, PA, USA).

2.9. Statistical analyses

Unless stated otherwise, the data are presented as mean±S.E.M. Comparisons between groups were assessed by two-tailed, unpaired student t-tests, and P values less than 0.05 were considered statistically significant.

3. Results

3.1. Time course of CK release after distal LAD ligation

Serum creatine kinase (CK) level corrected for total serum protein was studied in mice after permanent coronary occlusion (24, 72 h, and 7 days) along with time matched sham-operated controls (Fig. 1). Within the three different time points after distal LAD ligation, serum CK reached its highest level after the first 24 h (11.45±0.88 U/g protein) and returned to a level comparable to that of sham-operated controls after 72 h (3.71±0.33 U/g protein). These results suggest that in our distal LAD ligation model, maximum tissue injury occurred within the first 24 h after coronary artery ligation, and that there was no significant ongoing tissue damage after 72 h.

Fig. 1. Serum creatine kinase (CK) level after distal LAD ligation at 24, 72 h and 7 days and time-matched sham control. Serum CK (U/dl) was corrected by serum protein level (g/dl). The blood was obtained directly from LV cavity while the heart was still beating. The serum CK reached its highest level at the first 24 h, and then returned to the sham level after 72 h.

3.2. Infarct area measurement

To quantify the magnitude of tissue damage after the experimental left coronary artery ligation, the infarct area was measured and percent infarct size was calculated (Fig. 2). There was no discernible ventricular dilatation in the ligated group compared with the sham group (data not shown). The infarct size generated by the distal LAD ligation was significantly smaller than that reported by using the conventional high ligation method [11,14]. There was no significant difference in the infarct size at 24 h (n=10) and 72 h (n=9) after coronary ligation (14.5±1.2 and 14.3±0.6%, respectively), indicating that the majority of myocardial injury occurred within the first 24 h of myocardial ischemia, and that there was no obvious infarct expansion after 24 h.

3.3. Hemodynamic data after distal LAD ligation

Invasive in vivo hemodynamic evaluation via left ventricular catheterization was performed in mice 7 days after permanent myocardial ischemia by distal LAD ligation (n=13) and time-matched sham operated controls (n=8) (Table 2). Our data indicated that no significant secondary

Table 2

<table>
<thead>
<tr>
<th>Hemodynamic parameters</th>
<th>Sham (n=8)</th>
<th>LAD ligation (n=13)</th>
<th>t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart/body (mg/g)</td>
<td>5.26±0.15</td>
<td>5.86±0.16</td>
<td>P&lt;0.05</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>419±19</td>
<td>383±20</td>
<td>N.S.</td>
</tr>
<tr>
<td>LVSP (mmHg)</td>
<td>72.5±2.3</td>
<td>69.3±1.9</td>
<td>N.S.</td>
</tr>
<tr>
<td>LVEDP (mmHg)</td>
<td>7.4±1.2</td>
<td>6.6±0.8</td>
<td>N.S.</td>
</tr>
<tr>
<td>(+) dP/dt max</td>
<td>3543±170</td>
<td>3274±160</td>
<td>N.S.</td>
</tr>
<tr>
<td>(−) dP/dt max</td>
<td>3079±246</td>
<td>2900±133</td>
<td>N.S.</td>
</tr>
</tbody>
</table>
Table 3
Effects of dobutamine infusion

<table>
<thead>
<tr>
<th></th>
<th>Sham (n=5)</th>
<th>LAD ligation (n=6)</th>
<th>t test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart/body (mg/g)</td>
<td>5.26±0.21</td>
<td>6.15±0.21</td>
<td>P&lt;0.01</td>
</tr>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>389±14</td>
<td>398±11</td>
<td>N.S.</td>
</tr>
<tr>
<td>(+)dP/dt_max</td>
<td>3551±400</td>
<td>3536±289</td>
<td>N.S.</td>
</tr>
<tr>
<td>Dobutamine stress</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR change (%)</td>
<td>143±6</td>
<td>134±6</td>
<td>N.S.</td>
</tr>
<tr>
<td>(+)dP/dt_max change</td>
<td>214±9</td>
<td>205±11</td>
<td>N.S.</td>
</tr>
</tbody>
</table>

Hemodynamic alteration took place after distal LAD ligation and that there was no statistically significant difference between the two groups in heart rate (HR), left ventricular systolic pressure (LVSP), left ventricular diastolic pressure (LVEDP), (+)dP/dt_max and (-)dP/dt_max. The heart/body ratio, however, was clearly increased in the experimental group compared with the sham operated group (5.868±0.163 and 5.256±0.153 mg/g, respectively; P<0.05), suggesting that compensatory myocardial hypertrophy might have occurred in non-ischemic myocardial tissue after myocardial infarction. It should be noted that our results may be different from the hemodynamics in the awake condition since the study was performed under general anesthesia.

We also performed dobutamine stress test in both sham and LAD-ligated mice. As summarized in Table 3, there was no difference in HR and (+)dP/dt_max between sham and LAD ligation group before dobutamine challenge. Most importantly, infusion of dobutamine resulted in a comparable levels of increase in HR and (+)dP/dt_max in these two groups, suggesting that the functional response to stress was maintained in our LAD ligation group.

3.4. mRNA expression after small restrictive myocardial infarction

The temporal mRNA expression of selected markers for myocardial fibrosis was studied by semi-quantitative RT-PCR at 24, 72 h and 7 days after distal LAD ligation in both AAR and ANAR (Fig. 3). The markers examined included collagens (types I, III and VI), lysyl oxidase, and TGF-β1. Collagens type I and III are not only major components of the scar tissue in AAR but also are components of myocardial fibrosis in ANAR after MI [19,20]. Lysyl oxidase, a cross-linking enzyme for col-

![Fig. 3](A) Expression of mRNA for extracellular matrix proteins (collagens type I, III, and VI), lysyl oxidase and TGF-β1 in AAR and ANAR at 24, 72 h and 7 days after distal LAD ligation by semi-quantitative RT-PCR. (B) and (C) Bar graphs showing the quantitative expression of mRNAs after distal LAD ligation (normalized by GAPDH expression). Normalized values in sham-operated hearts are arbitrarily expressed as 1.0. The values of bar graphs are calculated from semi-quantitative RT-PCR in three different sample series, and are expressed as mean±S.E.M. Collagens type I, III, and VI are all up-regulated in AAR after 72 h. A similar pattern, though to a lesser extent, is seen in the ANAR. Lysyl oxidase shows the most prominent increase in AAR after 72 h. The substantial increase of lysyl oxidase in ANAR is seen only after 7 days. TGF-β1 is dramatically increased after 72 h in AAR, but a substantial increase is also seen in ANAR after 72 h (4–6-fold increase). All statistical analyses were performed in comparison with sham. * P<0.05, ** P<0.01, *** P<0.005.
lagens, mediates soluble collagen molecules into insoluble fibrous organization [21]. TGF-β1 is a key cytokine that up-regulates collagen gene expression. The results showed that collagens type I (α1 chain), type III (α1 chain), and type VI (α1 chain), began to increase after 72 h in both AAR (7–10-fold) and ANAR (3–7-fold). Lysyl oxidase was dramatically up-regulated after 72 h in AAR (26–33-fold). It was also up-regulated in ANAR after 7 days (8-fold). TGF-β1 was significantly up-regulated after 72 h in AAR (12–17-fold) and in ANAR (4–5-fold).

3.5. Spatio-temporal protein expression in ANAR after distal LAD ligation

Immunohistochemical analyses of collagens type I, III and VI were performed in AAR and ANAR at 72 h and 7 days after distal LAD ligation with sham myocardium as a control. As expected, these proteins were predominantly localized in the scar tissue in the AAR at 72 h and 7 days after induced myocardial infarction (results not shown). Significantly increased expression of these collagens was also found in the remote non-ischemic ANAR after 72 h (Fig. 4). After 72 h and 7 days, the expression of collagens type I and III, which was restricted to the perivascular regions in the normal non-ischemic myocardium (not shown), was diffusely up-regulated in the myocardial interstitial tissue (Fig. 4B,C,E,F). Collagen type VI, which was evenly expressed in the endomysium of the sham myocardium, also revealed a significant increase in the interstitial tissue after 72 h and 7 days.

4. Discussion

4.1. Myocardial fibrosis induced by small myocardial infarction

Large myocardial infarction (MI) can induce ventricular dilatation and subsequent ventricular dysfunction or congestive heart failure, both clinically [1,23] and ex-

Fig. 4. Double staining immunohistochemical study to assess protein localization in the remote non-ischemic myocardium (ANAR) after distal LAD ligation. A–C: collagen type I, D–F: collagen type III, and G–I: collagen type VI (all in red color). Green fluorescence indicates localization of PECAM-1, which outlines intermyocardial capillary endothelial cells. Collagen type I is not expressed in the sham myocardium (A), but its expression is substantially increased after 72 h (B) and 7 days (C) in the interstitial myocardium (see arrows). Collagen type III has similar expression pattern (D–F). Collagen type VI is expressed evenly along the endomysium of sham myocardium (G), but its intensity increases remarkably after 72 h and 7 days (H and I; see arrows). Magnification bar = 100 μm.
4.2. Pathological process after hemodynamically insignificant myocardial infarction

Significant increases in mRNAs for collagens type I and type III in AAR are detected 72 h and 7 days after the ligation, while in ANAR these increases are rather gradual (Fig. 3). A similar finding in the increases in mRNA expression of collagens type I and type III in the ischemic area compared to the non-ischemic areas was recently reported in the rat model after large myocardial infarction [8]. The increase in collagen type III mRNA occurred earlier than that of type I collagen (Fig. 3), consistent with the early appearance of type III collagen during wound healing [24]. In ANAR, these collagens not only increased in amounts in the myocardium, but their spatial localizations are different from those of the sham myocardium (Fig. 4). Overall, our findings on the expression of collagens I and III or development of myocardial fibrosis in the mouse model are similar to previous studies with experimentally induced large myocardial infarction in the rat [8,25].

In addition, our study shows that collagen type VI, which plays an important role in skin wound healing [26,27] and in pathological fibrosis of various organs [28,29], is up-regulated after experimental myocardial ischemia in AAR and ANAR with a time course similar to that of type III collagen. Type VI collagen, which is expressed evenly in the endomysium in the normal condition, shows significant up-regulation predominantly in the interstitial tissue of the myocardium in ANAR following small myocardial infarction. Its specific role in the ventricular remodeling is yet to be determined.

Our study further indicates that lysyl oxidase, a key enzyme catalyzing cross-links of collagens, is dramatically up-regulated in AAR after 72 h, which correlates well with actual collagen deposition and scar formation in the infarcted area. Marked up-regulation of lysyl oxidase is also evident in the ANAR, in accordance with the increase in collagen cross-linking previously reported in the non-ischemic myocardium following experimentally induced large myocardial infarction [30]. A previous study showed that inhibition of collagen cross-linking results in a reduction of fibrillar collagen deposition in the myocardium and thus a reduction in ventricular diastolic stiffness [31]. The up-regulation of lysyl oxidase in ANAR therefore also represents an early sign of myocardial fibrosis in the remote non-ischemic myocardium.

TGF-β1 was previously shown to be up-regulated in post-infarction ventricular remodeling and to be responsible for myocardial fibrosis and maladaptive extracellular matrix (ECM) remodeling [32,33]. Up-regulation of TGF-β1 expression was observed during the transition from stable hypertrophy to heart failure, which is thought to initiate increase interstitial fibrosis [34]. Our study shows that in AAR, TGF-β1 mRNA increases 24 h after distal ligation, reaches a maximum after 3 days and gradually decreases after 7 days (Fig. 3). In the ANAR, however, the TGF-β1 mRNA level continues to increase after 7 days. The time course of TGF-β1 induction in the AAR is again similar to that previously reported in the rat model after large myocardial infarction with secondary hemodynamic changes [8]. Although it is clear that TGF-β1 intermediates myocardial fibrosis in the remote non-ischemic myocardium, the underlying mechanism that regulates TGF-β1 is yet to be explored.

4.3. Post-ischemic ventricular remodeling as a spectrum of benign myocardial adaptation to progressive heart failure

Myocardial hypertrophy is commonly recognized as an adaptive response that normalizes wall stress and compensates for mechanical overload [12]. Ventricular remodeling is a complex gross geometric alteration in the microstructure of entire ventricular myocardium which common-
ly implies failure in the adaptation process [3, 4]. Although our model may be regarded as within the range of benign physiological adaptation in response to a limited degree of ischemic injury, the molecular expression of TGF-β1 and ECM proteins as well as disruption of myocardial microstructure is quite similar to that of ventricular remodeling following a large MI. Our observation in the small MI model has enabled us to propose a hypothesis that postischemic ventricular remodeling is a pathological spectrum ranging from benign myocardial adaptation to progressive heart failure, and that ischemic injury itself can induce this spectrum. The fate of affected heart, as an organ, is primarily affected by the magnitude of the secondary mechanical factors via other signaling pathways, such as the angiotensin II and endothelin I pathways [2, 20]. When considering possible treatment and/or prevention of postischemic heart failure, these initial molecular changes could be first important process for the later development of heart failure.

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