Prolonged myocardial ischemia leads to myocyte necrosis, which initiates a localized inflammatory response that catabolizes the cellular debris and initiates deposition of extracellular matrix (ECM) proteins. In addition to providing a vital structural framework for the three-dimensional organization of cells, the ECM determines the physical properties of biological tissues and acts as a dynamic microenvironment for cellular signaling. A number of cardiac diseases, including myocardial ischemia and reperfusion (I/R), are associated with qualitative and quantitative alterations in ECM proteins. Understanding ECM changes is important for identifying factors that tip the balance between favorable reparative remodeling following myonecrosis and adverse remodeling that leads to progressive ventricular dilatation and congestive heart failure.

Extracellular Matrix Proteomics in Cardiac Ischemia/Reperfusion
The Search Is On

Kristine Y. DeLeon, PhD; Lisandra E. de Castro Brás, PhD; Richard A. Lange, MD; Merry L. Lindsey, PhD

ECM proteomics is the comprehensive description of the ECM expressed in a tissue at the time of evaluation. This approach generates a global, integrated view of extracellular processes and networks at the protein level. The goal of ECM proteomics is to reconcile gene activation to a particular extracellular phenotype. The ability to examine multiple proteins simultaneously is a substantial improvement over previous ECM studies that simply evaluated the total concentration of single proteins (ie, collagen) without consideration of type (eg, type I versus III versus IV) or quality (full-length versus partially degraded collagen).

In this issue of Circulation, Barallobre-Barreiro and colleagues present an innovative extracellular matridomic approach for the analysis of ECM protein changes in a porcine I/R model. With the use of a novel proteomic method to enrich for ECM proteins, tissue from infarcted and border zone regions of the left ventricle (LV) were analyzed separately by liquid chromatography tandem mass spectrometry for peptide identification and compared with control animals without I/R. Using these techniques, they identified 139 ECM proteins in porcine hearts subjected to 2 hours of ischemia followed by 15 or 60 days of reperfusion. Of the 139 proteins, 15 were identified for the first time in cardiac ECM (Table). Whereas many of the ECM proteins catalogued are known to have roles in wound healing, fibrosis, tissue remodeling, ECM polymerization, collagen fibril organization, and cell–ECM interaction, many of the newly identified cardiac ECM proteins have heretofore been associated with cartilage homeostasis, which involves pathways not known to be relevant to cardiac remodeling. For example, cartilage intermediate layer protein 1 has previously been associated with chondrocytes and adipocytes but not with cardiac myocytes or fibroblasts.

In addition to identifying ECM proteins, the investigators performed a principal component analysis, which provides a visual display of regional and temporal differences in ECM post-I/R. This statistical method is useful in analyzing complex data sets to reveal hidden data trends by summarizing (but not clustering) the data. Using this analysis, the investigators show that for both soluble and insoluble protein fractions the ECM proteins identified in the I/R groups are distinct from the controls.

Examining ECM at the proteomic level has several advantages over examining it at the genomic level. In contrast to gene array studies that only reveal which genes are upregulated at the time of evaluation, ECM proteomics assesses proteins that have accumulated in the tissue over time. Proteomics also provides information regarding posttranslational protein modifications, which are highly abundant in ECM, and plasma and extracardiac cell-derived ECM proteins that are not regulated by cardiac genes and, therefore, not identified by gene array analysis. Hence, to fully understand cardiac remodeling following I/R, an assessment of ECM proteomics is essential.

A major challenge faced by investigators who study cardiac ECM is the detection and identification of low-abundance proteins at their physiological concentrations. For example, analysis of the entire LV in animal models of myocardial infarction (MI) via two-dimensional electrophoresis gels results in the identification of a preponderance of mitochondrial proteins and no ECM proteins, because cardiomyocytes in the noninfarcted region contribute to 90% of the tissue volume, and mitochondria make up 30% of the cardiomyocyte cell volume. Thus, the contribution of the ECM,
although prominent in the infarct scar, is minimized when the entire LV is analyzed. A similar obstacle is encountered when human myocardial samples are analyzed. To enhance detection of ECM, the infarct, which is devoid of cardiomyocytes, and noninfarcted regions can be analyzed separately. Using this approach, we previously demonstrated matrix metalloproteinase (MMP)-9 dependent changes in infarct ECM composition post-MI, with fibronectin being an in vivo substrate of MMP-9. Similarly, Barallobre-Barreiro et al demonstrated robust expression of fibronectin at day 15 post-I/R.

The study in this issue of Circulation is notable for several reasons. First, by using a sequential extraction approach, Barallobre-Barreiro and colleagues identified soluble, newly synthesized ECM and insoluble integral ECM components (eg, cross-linked collagen, proteoglycans, and glycoproteins), as well. In cataloging the ECM matridome at 15 and 60 days post-MI, they also provide candidate-soluble ECM components that may be detected in the plasma for monitoring the post-MI repair process to determine whether optimal cardiac remodeling is occurring. The catalogue also provides targets to explore for therapeutic intervention. Because repeated tissue sampling (ie, cardiac biopsy) to determine cardiac ECM composition is not feasible in the clinical setting, identification of novel plasma biomarkers and biosignatures for prognostication in the post-MI setting is essential. Examining the soluble fractions also adds an extra dimension to the ECM catalogue by providing information on proteins that may undergo proteolysis in the I/R setting. ECM fragments (designated as matricryptins) serve as potent stimulators of cell signaling, but information regarding their formation in I/R is limited.

Second, by performing real-time polymerase chain reaction on the same tissue specimens used for proteomic analysis, the investigators revealed a discordant regulation of protein and messenger RNA levels in the injury and border-zone regions; although gene expression for selected ECM proteins was similar in both regions, the corresponding protein levels were much higher in the infarct area. This underscores the varying, but complimentary, information obtained from genomic and proteomic analyses. That there was little overlap between the gene and protein levels also underscores the fact that the best ways to validate proteomic data are to use immunoblotting or ELISA methods to confirm protein level changes.

Third, by analyzing border and infarct regions at 15 and 60 days post-MI, the investigators provide spatial and temporal data on ECM scar composition changes in response to I/R. At 15 days post-MI, few ECM proteins changed significantly at the border zone, but more pronounced changes were observed within the infarct area. Of special interest, cartilage and bone-related proteins such as aggrecan and chondroadherin were present at day 60 but not 15 days post-I/R. By combining the protein signature of early- and late-stage remodeling with an analysis of protein network interactions, the investigators propose that transforming growth factor beta 1 plays a pivotal role in regulating ECM post-MI. However, it is interesting to note that even though several members of the transforming growth factor-signaling pathway were identified in their study, transforming growth factor beta 1 itself was not. Further studies are needed to establish the cause-and-effect relationships of the protein interactions identified.

Fourth, several of the novel cardiac ECM proteins identified in the porcine I/R model were validated in LV tissue from patients with ischemic cardiomyopathy obtained at the time of cardiac transplantation, which demonstrates the potential clinical application of their findings. Although their I/R model and end-stage ischemic cardiomyopathy represent different time points along the posts ischemia time continuum, the persistent increase in these particular ECM proteins may provide mechanistic clues on factors that influence the progression to adverse remodeling.

The study has several limitations. Despite reperfusion, the prolonged (ie, 2 hour) episode of ischemia caused irreversible myocardial damage. Therefore, this study examined the ECM

### Table. Newly Identified Proteins in the Post-I/R Cardiac ECM

<table>
<thead>
<tr>
<th>Accession No.</th>
<th>Name</th>
<th>Currently Known Functions</th>
</tr>
</thead>
<tbody>
<tr>
<td>AGRN_HUMAN</td>
<td>Agrin</td>
<td>Aggregation of acetylcholine receptors</td>
</tr>
<tr>
<td>ASPN_HUMAN</td>
<td>Asporin</td>
<td>Negative regulator of chondrogenesis</td>
</tr>
<tr>
<td>C1L1P1_HUMAN</td>
<td>Cartilage intermediate layer protein 1</td>
<td>Cartilage scaffolding</td>
</tr>
<tr>
<td>COLA2_HUMAN</td>
<td>Collagen alpha-2(IV)</td>
<td>Structural integrity of basement membrane</td>
</tr>
<tr>
<td>COCA1_HUMAN</td>
<td>Collagen alpha-1(XII)</td>
<td>Mediates interaction between collagen fibrils and other ECM proteins</td>
</tr>
<tr>
<td>COEA1_HUMAN</td>
<td>Collagen alpha-1(XIV)</td>
<td>Integrates collagen bundles</td>
</tr>
<tr>
<td>EMIL1_HUMAN</td>
<td>EMILIN-1</td>
<td>Elastogenesis and maintenance of vascular cell morphology</td>
</tr>
<tr>
<td>MATN4_HUMAN</td>
<td>Matrilin-4</td>
<td>Formation of filamentous networks</td>
</tr>
<tr>
<td>NID1_HUMAN</td>
<td>Nidogen-1</td>
<td>Connects network of collagens and laminins</td>
</tr>
<tr>
<td>PODN_HUMAN</td>
<td>Podocan</td>
<td>Growth and migration of cells in cardiovascular tissues</td>
</tr>
<tr>
<td>PRELP_HUMAN</td>
<td>Prolargin</td>
<td>Anchoring basement membranes to underlying connective tissue</td>
</tr>
<tr>
<td>SPON1_HUMAN</td>
<td>Spondin-1</td>
<td>Axon outgrowth and cell migration during development</td>
</tr>
<tr>
<td>TENX_HUMAN</td>
<td>Tenascin-X</td>
<td>Matrix maturation during wound healing</td>
</tr>
<tr>
<td>TENN_HUMAN</td>
<td>Tetraneectin</td>
<td>Fibrinolysis and proteolysis during tissue remodeling</td>
</tr>
<tr>
<td>TINAL_HUMAN</td>
<td>Tubulointerstitial nephritis antigen-like</td>
<td>Promotes cell adhesion and spreading</td>
</tr>
</tbody>
</table>

I/R indicates ischemia and reperfusion; ECM, extracellular matrix.
changes following reperfusion of infarcted, rather than ischemic, myocardium. Whether similar ECM changes occur in myocardium, that is, (a) ischemic but viable or (b) infarcted without reperfusion remains to be tested. Because of the cost and complexity of the study, the analysis was limited to 2 time points (15 and 60 days post-I/R) analyzed in a relatively small number of animals. A more thorough evaluation of early ECM changes is needed to understand the interplay between the inflammatory and fibrotic responses and to identify appropriate prognostic and therapeutic targets. For example, MMP-2 was the only MMP identified in the ECM matridome, whereas other MMPs known to be involved in early post-MI remodeling, including MMP-3, -7, and -9, were not identified.

One caveat of the procedure used by the investigators for ECM enrichment is that this protocol does not likely solubilize the entire ECM, thereby providing an incomplete representation of ECM composition. Future studies using a solubilization buffer that completely dissolves the ECM into constituent components may better inform how cross-linked ECM is modified during the post-I/R response. Along these same lines, the investigators did not analyze cardiac tissue in the noninfarcted remote region of hearts subjected to I/R. Hence, it is unknown if the ECM changes (a) are the result of local signals and hence restricted to the infarct and border zones or (b) occur diffusely throughout the myocardium and result from hemodynamic (ie, LV wall stress) or systemic neurohormonal activation (ie, the renin-angiotensin and sympathetic nervous system). Finally, the animals studied did not receive drugs that are routinely administered to patients post-MI (ie, angiotensin-converting enzyme inhibitors, β-adrenergic blockers, statins, antiplatelet agents, and aldosterone inhibitors), many of which are known to affect cardiac remodeling and may alter the ECM composition.

In conclusion, the in-depth analysis of the cardiac ECM response to I/R by Barallobre-Barreiro et al provides candidate biomarkers of remodeling that extend our list beyond collagen. Future animal studies using experimental conditions, such as genetic deletions in one (or more) components of pathways thought to be important in ECM regulation may provide a more comprehensive understanding of ECM involvement in cardiac remodeling. The information gained from ECM matridomic studies should continue to advance our understanding on how to prevent, or at least diagnose, progressive cardiac remodeling that will transition to heart failure.

Sources of Funding
This work was supported by NIH NHLBI T32 HL07446 (to K.Y.D.); the Rapoport Foundation for Cardiovascular Research (to R.A.L.); and NHLBIHHSN 268201000036C (N01-HV-00244) for the UTHSCSA Cardiovascular Proteomics Center and R01 HL075360, the Max and Minnie Tomerlin Voelcker Fund, and the Veteran’s Administration (Merit) (to M.L.L.).

Disclosures
None.

References

Key Words: Editorials ■ extracellular matrix proteomics ■ extracellular matrix ■ ischemia-reperfusion ■ proteomics
Extracellular Matrix Proteomics in Cardiac Ischemia/Reperfusion: The Search Is On
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Circulation. 2012;125:746-748; originally published online January 18, 2012;
doi: 10.1161/CIRCULATIONAHA.111.086835
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

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