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GLYCOPROTEOMICS ANALYSIS REVEALS A REDUCTION OF DECORIN FRAGMENTS WITH ANTI-MYOSTATIN ACTIVITY IN ATRIAL FIBRILLATION

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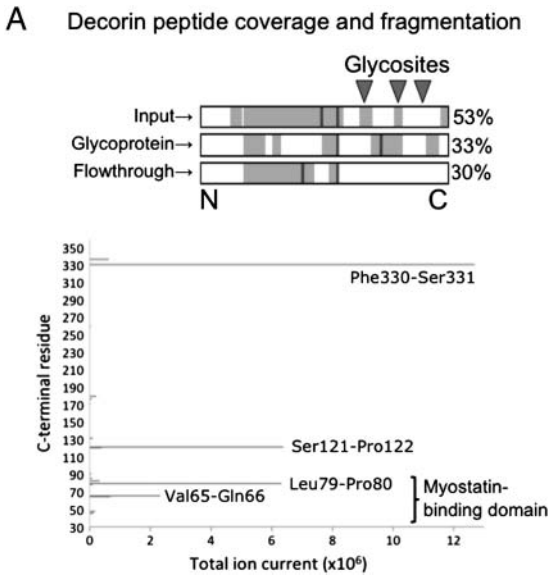
Background A comprehensive characterisation of the cardiac extracellular matrix (ECM) is pertinent for a better understanding of cardiovascular disease processes. We have recently established a novel proteomic method to profile cardiac ECM proteins and applied it to a porcine model of ischemia-reperfusion injury (Barallobre-Barreiro et al, Circulation, 2012). In the present study, we analyzed human atrial tissue.

Methods Left and right atrial specimen were obtained from five patients undergoing coronary bypass grafting. ECM proteins were enriched using a sequential extraction procedure followed by affinity capture of glycoproteins using concanavalin-A and wheat germ agglutinin before analyses by mass spectrometry.

Results Few ECM-related glycoproteins, such as lumican, fibulin-2, latent TGFβ-binding protein 4 and clusterin were differentially expressed in left and right atria. Intriguingly, one glycoprotein was consistently identified in the non-glycosylated flow-through: decorin, a member of the small leucine-rich proteoglycan family (SLRPs), has three glycosylation sites in the C-terminal half of the protein. Only N-terminal fragments were detected in the flow-through. Mass spectrometry analyses of non-tryptic cleavage sites provided unambiguous evidence for decorin fragmentation in human atrial tissue. In contrast, other SLRPs, such as biglycan, were barely fragmented despite a high degree of sequence homology. Amongst the four most abundant decorin cleavage products were peptides with known anti-myostatin activity. Myostatin is a potent negative regulator of muscle growth. The mass spectrometry findings were confirmed using peptide-specific antibodies. Further analyses of atrial samples from patients with long-standing atrial fibrillation (AF) revealed that levels of full-length decorin increased during structural remodeling in AF. Its cleavage products with anti-myostatin activity, however, were reduced compared to atria from patients in sinus rhythm.

Conclusions For the first time, cleavage products of decorin with known anti-myostatin activity were identified in human cardiac



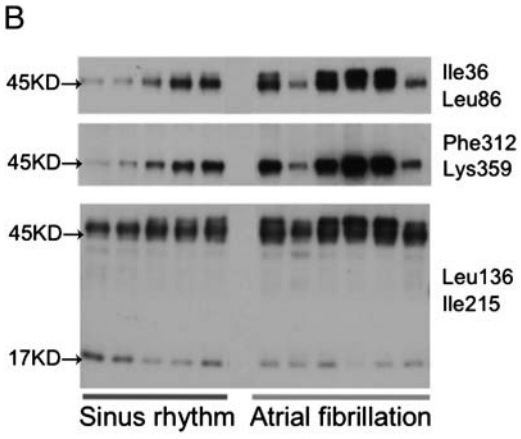


Figure 1 A) Sequence coverage for decorin in the input, the glycoprotein-enriched and the flow-through fraction. No C-terminal peptides were detected in the flow-through (upper panel). Abundance of decorin cleavage sites according to ion intensities in mass spectrometry. Myostatin-binding domains are highlighted (bottom panel). B) Cleavage of decorin as determined by antibodies raised against the N-terminal (Ile36-Leu86), C- terminal (Phe312-Lys359) and internal domains (Leu136-Ile215). Note that increased expression of full-length decorin (45KD) in AF is associated with a reduction of its proteolytic fragments.

tissue and shown to be reduced in AF. The presence of these myostatin regulators could have important implications for the growth factor response in cardiac disease.