Human pericardial proteoglycan 4 (lubricin): Implications for postcardiotomy intrathoracic adhesion formation

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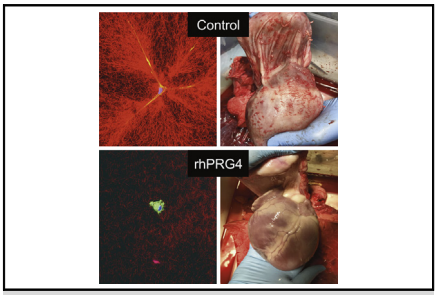
ABSTRACT

Objective: Intrapericardial fibrous adhesions increase the risk of sternal reentry. Proteoglycan 4/lubricin (PRG4) is a mucin-like glycoprotein that lubricates tissue compartments and prevents inflammation. We characterized PRG4 expression in human pericardium and examined its effects in vitro on human cardiac myofibroblast fibrotic activity and in vivo as a measure of its therapeutic potential to prevent adhesions.

Methods: Full-length PRG4 expression was determined using Western blot analysis and amplified luminescent proximity homogeneous assay in human pericardial tissues obtained at cardiotomy. The in vitro effects of PRG4 were investigated on human cardiac myofibroblasts for cell adhesion, collagen gel contraction, and cell-mediated extracellular matrix remodeling. The influence of PRG4 on pericardial homeostasis was determined in a chronic porcine animal model.

Results: PRG4 is expressed in human pericardial fluid and colocalized with pericardial mesothelial cells. Recombinant human PRG4 prevented human cardiac myofibroblast attachment and reduced myofibroblast activity assessed using collagen gel contraction assay (64.6% ± 8.1% vs 47.1% ± 6.8%; $P = .02$). Using a microgel assay, human cardiac myofibroblast mediated collagen fiber remodeling was attenuated by PRG4 (1.17 ± 0.03 vs 0.90 ± 0.05; $P = .002$). In vivo, removal of pericardial fluid alone induced severe intrapericardial adhesion formation, tissue thickening, and inflammatory fluid collections. Restoration of intrapericardial PRG4 was protective against fibrous adhesions and preserved the pericardial space.

Conclusions: For the first time, we show that PRG4 is expressed in human pericardial fluid and regulates local fibrotic myofibroblast activity. Loss of PRG4-enriched pericardial fluid after cardiotomy might induce adhesion formation. Therapeutic restoration of intrapericardial PRG4 might prevent fibrous/inflammatory adhesions and reduce the risk of sternal reentry. (J Thorac Cardiovasc Surg 2018;■:1-11)



rhPRG4 attenuates human fibroblast fibrotic activity and prevents pericardial adhesions.

Central Message

Full-length PRG4 is documented in human pericardial fluid and shown in vitro to regulate human myofibroblast activity. In vivo, rhPRG4 prevented intrapericardial fibrous adhesion formation.

Perspective

Postoperative intrapericardial adhesions increase reoperative risk. We show that PRG4 is expressed in human pericardial fluid and regulates human fibroblast activity. Removal of pericardial fluid induced severe adhesion formation whereas PRG4 replacement was protective. Restoring intrapericardial PRG4 after cardiac surgery might attenuate postoperative adhesions and increase the safety of reoperation.

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Postoperative retrosternal pericardial adhesion formation is a consequence of inflammatory and fibrotic responses after tissue injury. Adhesions are associated with greater mortality risks and risk of injury for patients who undergo sternal reentry.[1] Previously reported complications include damage to previous bypass grafts, the heart, and innominate vessels,

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Abbreviations and Acronyms

AlphaLISA = amplified luminescent proximity homogeneous assay
DAPI = 4′,6-diamidino-2-phenylindole
ECM = extracellular matrix
mAb = monoclonal antibody
PBS = phosphate buffered saline
PRG4 = proteoglycan 4/lubricin
rhPRG4 = recombinant human proteoglycan 4 (lubricin)
SDS-PAGE = sodium dodecyl sulfate polyacrylamide gel electrophoresis
SMA = smooth muscle actin
TGF = transforming growth factor

among others.[2] Despite these well documented risks, there is no widely accepted and validated therapy in contemporary surgical practice to prevent or attenuate postsurgical intrapericardial adhesion formation.

During cardiac surgery, denudation of the pericardial mesothelium from serous pericardial layers creates a pathologic condition facilitating the formation of a fibrin mesh network.[3] Fibrin mesh creates a platform by which proinflammatory and profibrotic cytokines, such as transforming growth factor-β1 (TGF-β1), can increase myofibroblast migration and activation.[4,5] Increased α-smooth muscle actin (α-SMA) protein expression in myofibroblasts has been shown in surgical adhesions suggesting increased cellular activity and a profibrotic response.[6] Myofibroblast activation and extracellular matrix (ECM) protein production and remodeling are important mechanisms by which mild adhesions transition to dense, fibrous adhesion requiring sharp dissection during sternal reentry.[3,6,7]

Lubricin, also known as proteoglycan 4 (PRG4), is a mucinous glycoprotein found endogenously in numerous tissue compartments with diverse biological functions, of which joint synovial fluid is the best known example.[8,9] Lubricin/PRG4 has anti-inflammatory properties and has been shown to influence fibroblast adhesion and proliferation.[10-13] Administration of recombinant human PRG4 (rhPRG4) in a rat model of intra-abdominal adhesions was shown to limit the development of postoperative adhesions.[14] We and others speculate that loss of PRG4 might induce adhesion formation and accordingly, restoring lost PRG4 within tissue compartments might help prevent postsurgical adhesion formation.

PRG4 expression in the human pericardial mesothelium tissue was recently documented through immunohistochemistry; however, expression of full-length PRG4 has not been confirmed.[15] We therefore sought to further explore expression of PRG4 in human pericardial tissues as well as its fluid. In addition, we explored the biological effects of rhPRG4 on human cardiac myofibroblast activity and cell-mediated ECM remodeling in vitro. Furthermore, we assessed the in vivo effects of pericardial fluid loss and its restoration with rhPRG4 in a preclinical porcine model to provide proof-of-concept data.

METHODS

Recombinant Human Lubricin/PRG4

Purified rhPRG4 solution was obtained from Lubris BioPharma, LLC (Framingham, Mass). The rhPRG4 was generated and purified as described previously.[10-12]

Human Tissue Procurement

Pericardial fluid and tissue samples were obtained from consenting patients undergoing cardiac surgery at Foothills Medical Centre (Calgary, Alberta, Canada). Experiments involving human tissue usage were approved by the Conjoint Health Research Ethics Board at the University of Calgary and conform to the Declaration of Helsinki.

Western Blot Analysis

Western blot analysis to detect PRG4 protein in pericardial fluid samples were performed as previously described.[16] Briefly, samples were subjected to Novex 3-8% Tris-Acetate sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Blotted membranes were incubated overnight with anti-PRG4 monoclonal antibody (mAb) 9G3 (EMD Millipore, Billerica, Mass) at 1:50,000 (20 ng/mL), washed, then treated with secondary antibody goat anti-mouse horseradish peroxidase (SigmaAldrich, St Louis, Mo) at 1:1000. Immunoreactive bands were detected using enhanced chemiluminescence substrate (SuperSignal West Femto; ThermoScientific, Waltham, Mass) and a gel imaging system (ImageQuant LAS 4000; GE, Toronto, Ontario, Canada).

Amplified Luminescent Proximity Homogeneous Assay Quantification of Full-Length PRG4

PRG4 was quantitatively determined using amplified luminescent proximity homogeneous assay (AlphaLISA; Perkin Elmer, Boston, Mass) technology. Briefly, streptavidin modified donor beads were bound to biotin-labeled rhPRG4, and protein G modified acceptor beads were bound to anti-PRG4 mAb 9G3. Competitive inhibition of the rhPRG4–9G3 interaction by free unlabeled PRG4 in solution was used for PRG4 quantification (Appendix E1).

Immunohistochemistry

Pericardial tissue samples were harvested from cardiac surgery patients and fixed in 4% paraformaldehyde for 15 minutes.[17] After incubation in mAb 9G3 at 1:200 dilution in 1.5% goat serum phosphate buffered saline (PBS) overnight at 4°C, sections were washed in PBS and incubated with Alexa-Fluor 594 rhodamine-conjugated goat anti-mouse immunoglobulin G secondary antibody (Life Technologies, Carlsbad, Calif) at a dilution of 1:100. Sections were mounted with 4′,6-diamidino-2-phenylindole (DAPI) stain (Vectashield; Vector Laboratories, Burlingame, Calif). Samples were imaged using confocal microscopy.

Human Cardiac Myofibroblast Cell Isolation

As previously described, right atrial appendage biopsy samples were rinsed in phosphate buffered saline (PBS; Lonza, Walkersville, MD).[18,19] Tissues were minced and explant cultured with Iscove's Modified Dulbecco's medium (IMDM) supplemented with 10% fetal bovine serum (Gibco Life Technologies, Burlington, Ontario, Canada) and 5% penicillin-streptomycin (Life Technologies, Burlington, Ontario, Canada).