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The Proteoglycan Biglycan Enhances Antigen-Specific T Cell Activation Potentially via MyD88 and TRIF Pathways and Triggers Autoimmune Perimyocarditis

Zoran V. Popovic,* Shijun Wang,* Maria Papatriantafyllou,† Ziya Kaya,‡ Stefan Porubsky,* Maria Meisner,* Mahnaz Bonrouhi,* Sven Burgdorf,§ Marian F. Young,‖ Liliana Schaefer,‖ and Hermann-Josef Gröne*

Biglycan is a proteoglycan ubiquitously present in extracellular matrix of a variety of organs, including heart, and it was reported to be overexpressed in myocardial infarction. Myocardial infarction may be complicated by perimyocarditis through unknown mechanisms. Our aim was to investigate the capacity of TLR2/TLR4 ligand biglycan to enhance the presentation of specific Ags released upon cardiomyocyte necrosis. In vitro, OVA-pulsed bone marrow-derived dendritic cells from wild-type (WT; C57BL/6) and TLR2−/−, TLR4−/−, MyD88−/−, or TRIF-deficient mice were cocultured with LPS, biglycan, or vehicle and incubated with OVA-recognizing MHC I- or MHC II-restricted T cells. Biglycan enhanced OVA-specific cross-priming by >80% to MHC I-restricted T cells in both TLR2- and TLR4-pathway–dependent manners. Accordingly, biglycan-induced cross-priming by both MyD88−/− and TRIF-deficient dendritic cells (DCs) was strongly diminished. OVA-specific activation of MHC II-restricted T cells was predominantly TLR4 dependent. Our first in vivo correlate was a model of experimental autoimmune perimyocarditis triggered by injection of cardiac Ag-pulsed DCs (BALB/c). Biglycan-treated DCs triggered perimyocarditis to a comparable extent and intensity as LPS-treated DCs (mean scores 1.3 ± 0.3 and 1.5 ± 0.4, respectively). Substitution with TLR4-deficient DCs abolished this effect. In a second in vivo approach, WT and biglycan-deficient mice were followed 2 wk after induction of myocardial infarction. WT mice demonstrated significantly greater myocardial T lymphocyte infiltration in comparison with biglycan-deficient animals. We concluded that the TLR2/4 ligand biglycan, a component of the myocardial matrix, may enhance Ag-specific T cell priming, potentially via MyD88 and TRIF, and stimulate autoimmune perimyocarditis. The Journal of Immunology, 2011, 187: 6217–6226.
ments of the innate immune system and an obligatory part of an immune response to tissue damage. They exhibit a strong capacity to ingest external Ags via receptor-independent pinocytosis or receptor-mediated endocytosis, process them through MHC class II (MHC II) compartments, and present the Ags to induce CD4\(^+\) T cell responses (39, 40). Presentation of peptide Ag within MHC class I (MHC I) molecules will lead to recognition by naive CD8\(^+\) T cells. A specific subset of DCs characterized by a CD11c\(^+\)CD8\(\alpha\)\(^+\) phenotype demonstrates a capacity to present phagocytosed Ags within MHC I molecules, thus permitting an effective CTL response to tumor and viral Ags of extracellular origin (41–48). The phenomenon of CTL priming with externally acquired Ag was originally termed cross-priming, and the matching Ag-processing pathway in the DC is termed cross-presentation (49, 50). Maturation and activation of MΦs and DCs, classically coupled to the ingestion of microbial particles or endogenous danger signals, may trigger a set of immune-defense pathways. These include TLR signaling, as well as activation of MΦ-inducible C-type lectin and C-type lectin domain family 9 member A pathways through cell damage-associated molecules released from necrotic cells (38, 51–55). In this study, we hypothesized that biglycan acts as a TLR2/4 ligand to trigger and exacerbate an autoimmune response. The aim of this study was to evaluate the role of biglycan in Ag-specific T cell activation. We analyzed whether biglycan had the capacity to induce experimental autoimmune perimyocarditis (EAP) and influence T cell inflammation after myocardial infarction. Our in vitro results showed that biglycan clearly facilitated MHC I and MHC II (OTI and OTII)-restricted T cell priming, without having an effect on OVA uptake. The increase in cross-priming was TLR2 and TLR4 dependent and required both TLR2 and TLR4 adaptors MyD88, TRIF, and TIRAP, whereas MHC II-mediated Ag-specific T cell activation was mainly mediated by TLR4. In an in vivo EAP model, biglycan triggered myosin H chain \(\alpha\) (MyHC-\(\alpha\)) and troponin I (TnI)-dependent EAP comparable to the severity to LPS-triggered disease, acting in a TLR4-dependent manner. In a myocardial infarction model, pericardial and myocardial T cell infiltration 2 wk after induction of myocardial infarction was significantly stronger in control mice (C57BL/6) compared with biglycan-deficient mice. Both approaches strongly indicated that biglycan, a known endogenous TLR2/TLR4 ligand, may act as an inducer of pericardial and myocardial inflammation in injured heart tissue.

Materials and Methods

Mice

C57BL/6 and BALB/cJ wild-type (WT) mice were purchased from Charles River Germany. MyD88\(^{−/−}\), TRIF\(^{−/−}\), and OTI mice (C57BL/6) were bred and obtained from the German Cancer Research Center Animal Facility.
TLR2<sup>−/−</sup> mice on the C57BL/6 background and TLR4<sup>−/−</sup> mice on the C57BL/10 background (C57BL/6 and C57BL/10 share the same MHC H-2b haplotype) were provided by L.S., biglycan<sup>−/−</sup> mice (C57BL/6) were obtained from M.F.Y., and OTI mice (C57BL/6) were obtained from S.B. TLR4<sup>−/−</sup> (BALB/cAnPrt) and corresponding WT control (BALB/cByJ) mice were purchased from The Jackson Laboratory, Bar Harbor, ME. All mice were kept in specific pathogen-free conditions and used in accordance with local experimental guidelines.

**Cells, culture media, Abs, and reagents**

B3Z cells were obtained from the Tumor Bank of the German Cancer Research Center (56). OTI and OTII cells were magnetically isolated from the spleens of OTI and OTII mice using MACS technology and magnetically labeled using anti-CD90 Abs, according to the manufacturer’s protocol (Miltenyi Biotec, Bergisch Gladbach, Germany). DCs were cultured in complete IMDM (Invitrogen, Darmstadt, Germany) conditioned with 30% medium from GM-CSF–producing NIH 3T3 cells (R1). The B3Z, OTI, and OTII cells were cultured in RPMI 1640 (Sigma-Aldrich, Hamburg, Germany). Both media were conditioned with 10% heat-inactivated FCS, 100 IU/ml penicillin, 100 mg/ml streptomycin, 2 mM L-glutamine, 10 mM HEPES, and 50 mM 2-ME (all from Invitrogen). Endotoxin-free OVA was purchased from Hyglos (Bernried am Starnberger See, Germany). LPS (Escherichia coli, 0127:B6) was from Sigma. TIRAP inhibitory peptide set was purchased from Imgenex (San Diego, CA). Purified rat anti-mouse CD40 Ab (clone 3/23), allophycocyanin hamster anti-mouse CD11c (clone HL3), BD flex-sets (IL-2, IFN-γ), and BD Inflammation Kit were from BD PharMingen (Heidelberg, Germany). FITC anti-mouse MHC II (clone M5/114.15.2) was purchased from NatuTec (Frankfurt am Main, Germany). CFSE, OVA:Alexa Fluor 647 and Alexa Fluor 546 phalloidin were from Invitrogen. Goat anti-mouse CD3-e (M-20) Ab was from Santa Cruz Biotechnology (Heidelberg, Germany). Rat anti-mouse ER-HR3 Ab was purchased from Acris Antibodies (Herford, Germany).

**Purification of human biglycan**

Human biglycan was expressed in 293 HEK cells, as described previously (57). For purification of native biglycan, the conditioned media were supplemented with protease inhibitors (0.1 M e-aminocaproic acid, 10 mM EDTA, 5 mM benzamidine, 10 mM N-ethylmaleimide, 1 mM phenylmethyl sulfonyl fluoride) and passed over a DEAE-Trisacryl-M (Pall) column, followed by elution with 20 mM Tris/HCl (pH 7.4), containing 1 M NaCl. After concentrating the relevant fractions with Aquacide I, as instructed by the manufacturer (Calbiochem), biglycan was dialyzed for 2 h and subsequently cocultured with B3Z, OTI, or OTII T cells (64). In some experiments, DCs were pretreated with TIRAP inhibitory peptide (DRIKIQWFQRMRKWKLLRDAAPGGGVS), as described (65). To quantify T cell activation, supernatants were removed after 18 h of coculture and assayed by BD CBA Flex-Set for IL-2 (B3Z) or IFN-γ (OTI and OTII) using a FACS Calibur. To directly evaluate T cell proliferation, OTI and OTII T cells were labeled for 10 min with 5 μM CFSE, thoroughly washed, and cocultured with BMDCs for 48 or 96 h. Proliferation was determined by flow cytometric analysis of the CFSE dilution (66).

**In vivo murine EAP assay and myocardial infarction model**

Immature BMDCs from WT or TLR4<sup>−/−</sup> mice (BALB/c) were pulsed for 1 h with 10 μg/ml mouse MyHC-α (614–629), 5 μg/ml CD40-stimulating Ab (CD40L), and 1 μg/ml LPS (positive control mice)

**Generation of bone marrow-derived DCs**

Bone marrow-derived DCs (BMDCs) were generated using GM-CSF–rich medium from R1 cells, as described (58, 59). At day 7, the DCs were checked for CD11c and MHC II expression (FACS analysis detected >90% of cells CD11c<sup>MHC II</sup>) and used in the experiments.

**Light microscopy, morphometric analyses, confocal microscopy, and flow cytometry**

Image acquisition and analysis were done using a Leica TCS-SL microscope and Leica confocal software (v. 2.6).1. Morphometry of the acute experimental perimyocarditis model was performed on 5-μm H&E-stained heart sections (three sections/mouse). The degree of inflammation was assessed using a 0–4 scoring scale from previously published models (60–62): 0, no inflammation; 0.5, focal inflammation; 1, <10%; 2, 11–30%; 3, 31–50%; and 4, 51–90% leucocyte infiltration within a heart section in which only ventricles were analyzed. Morphometric analysis in the myocardial infarction model was performed using a semiautomatic image-analyzing system (Leica Q600 QWin, Cambridge, U.K.) (63). The area of postinfarction damage (fibrosis and granulation tissue) was determined on 5-μm H&E- and Masson’s trichrome-stained heart sections. Five heart sections/mouse were evaluated. Results were expressed as the percentage damage of the total heart tissue present on the section, obtained after exclusion of atria and major blood vessels. Analyses were performed independently by two morphologists (H.-J.G. and Z.V.P.) in a blinded manner. Flow cytometric acquisition and analysis were performed using a FACS-CALIBUR flow cytometer and BD CellQuest Pro v5.2.1 and FCAP Array v1.0.1. software (BD Biosciences, Heidelberg, Germany).
Alternatively, MyHC-α was replaced with 10 μg/ml mouse TnI peptide (61, 62). In some experiments, biglycan (2 μg/ml) was applied either instead of LPS (as a TLR ligand) or instead of MyHC-α/TnI (to substitute for the Ag). Control BMDCs were pulsed with biglycan and CD40L only. Activated DCs were immediately injected i.p. in WT or TLR4−/− mice at a concentration of 0.25 × 10^6/0.5 ml medium/mouse (day 0). The remaining DCs were frozen at −80°C and thawed at days 2 and 4 for reinjection. The mice were euthanized, and hearts, spleens, kidneys, and lungs were removed for analyses at day 8. Myocardial infarction was achieved by permanent ligation of the left anterior descending artery, as described (68). Animals that survived the acute-infarction phase were sacrificed on day 14, and hearts were removed for analysis.

**Histology and immunohistology**

Five-micrometer serial sections were made from paraffin-embedded hearts and stained with H&E, to detect the inflammatory mononuclear cell infiltration, or Masson’s trichrome staining, to determine the fibrosis, as described. Immunohistochemical staining of mouse heart tissue sections for biglycan with chicken anti-rat MAY-01 Ab (1:500) was performed in 2-μm-thick heart tissue sections fixed with 4% formaldehyde in PBS, as described previously (69). Immunohistochemical stainings of formalin-fixed paraffin-embedded heart sections with purified rat anti-mouse CD4

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**FIGURE 3.** Cross-priming and MHC II-mediated T cell priming. After coincubation with OVA (1 mg/ml), OVA.Bgn, or OVA.LPS for 2 h, BMDCs were washed and cocultured with B3Z, OTI, or OTII cells. A, Cross-priming of B3Z cells by OVA. Statistical analysis of IL-2 secretion by B3Z cells after overnight coculture with BMDCs stimulated with control OVA (+ vehicle), OVA.Bgn, or OVA.LPS. Data are mean ± SEM from three experiments in triplicates. Statistical differences were evaluated by the paired t test. **p < 0.01, ***p < 0.001. B and C, Cross-priming and MHC II-mediated priming of OTI and OTII cells by OVA. T cells were isolated from the spleens of OTI and OTII mice, sorted by CD90.2 magnetic beads, and labeled by CFSE, as described. T cells were coincubated with OVA-presenting BMDCs for 48 and 96 h (OTI, B) or 96 h (OTII, C). Representative graphs from two experiments in duplicates are shown.
Statistical analyses

Efficient uptake of OVA by BMDCs is not altered by biglycan or LPS stimulation

To analyze the uptake of OVA by BMDCs, we incubated DCs from C57BL/6 mice with fluorescence-labeled OVA (OVA:Alexa Fluor 647) together with biglycan, LPS, or vehicle. After 15 min of coinoculation, DCs were subjected to confocal (Fig. 1A) and flow
cytometric (Fig. 1B) evaluation of intracellular fluorescence intensity. Statistical analyses (Fig. 1C) did not show significant differences in the intensity of OVA uptake among the control, biglycan-treated, and LPS-treated groups. To confirm previously described TLR2 and TLR4 receptor activation by biglycan, we incubated BMDCs with biglycan, LPS, or vehicle for 12 h and analyzed the supernatant for TLR-dependent inflammatory cytokines. Our results showed abundant TNF, IL-6, and MCP-1 secretion after stimulation with biglycan or LPS. Blockade of the MyD88 pathway almost completely prevented the secretion of proinflammatory cytokines, whereas TRIF pathway blockade induced a significant reduction in biglycan- and LPS-induced cytokine secretion (Fig. 2).

Biglycan enhances OVA-specific activation of B3Z, OTI, and OTII cells

To further investigate the effect of biglycan on Ag-specific T cell activation, we incubated OVA-loaded DCs with OVA-recognizing MHC I-restricted T cell hybridoma (B3Z) and ex vivo-isolated MHC I and MHC II (OTI and OTII) T cells. B3Z activation was evaluated by measurement of secreted IL-2 from the cell supernatant, whereas OTI and OTII cell proliferation was determined using CFSE labeling. Our results showed that costimulation of DCs with OVA and biglycan significantly increased OVA-specific activation of B3Z cells (Fig. 3A). Accordingly, cross-priming of OTI cells was strongly induced in biglycan- and LPS-treated groups in an increasing manner (after 48 and 96 h of DC/OTI

Figure 6. In vivo induction of EAP. Photomicrographs of BALB/c mice heart sections. A, BMDCs developed from BALB/c mice were incubated with the following cocktails: TnI+LPS+CD40L (upper left panel), TnI+biglycan+CD40L (upper right panel), MyHC-a+LPS+CD40L (lower left panel), or MyHC-a+biglycan+CD40L (lower right panel) (original magnification ×100). B, Heart inflammation was scored as 0–4, based on the percentage of the heart section affected by leukocyte infiltration (0, no inflammation; 0.5, focal inflammation; 1, <10%; 2, 11–30%; 3, 31–50%; and 4, 51–90%). **p < 0.01. C, Stained heart sections were analyzed for CD3+ and CD4+ T cell infiltration (original magnification ×200). CD3+ cells were counted in three high-power fields (HPF; ×200)/mouse. Data in B and C are mean ± SEM of three experiments in triplicates. Statistical differences were evaluated using the paired t test. D, Photomicrographs of heart sections from control mice (left to right): injection of DCs stimulated by cardiac-specific Ag without TLR ligand, Bgn without cardiac Ag, and Bgn and LPS without cardiac Ag (H&E, original magnification ×200). Representative photomicrographs from three experiments in triplicates are shown.
Deficient BMDCs, injected into either WT or TLR4−/− mice, induced a pattern and intensity of biglycan-induced stimulated DCs. In the case of BMDCs from WT mice administered to WT mice, the pattern and intensity of biglycan-induced stimulated DCs was modulated by TLR4 in a dose-dependent manner. Although biglycan-induced MHC II-mediated Ag presentation preferentially via the TLR4 pathway.

To investigate the influence of TLR2 and TLR4 on biglycan-mediated enhancement of Ag presentation, BMDCs from TLR2−/− and TLR4−/− mice were included in the experiments (Fig. 4). Biglycan-induced cross-priming of OVA to B3Z cells was completely abrogated in TLR2−/− and TLR4−/− DCs (Fig. 4A). As expected, LPS-triggered Ag presentation to B3Z cells was blocked in TLR4−/− DCs, and it remained nearly intact in TLR2−/− DCs (Fig. 3A). The same phenomenon was observed with regard to biglycan- or LPS-induced cross-priming of OTII cells (Fig. 4B). However, biglycan-induced MHC II-mediated priming of OTII cells appeared to be predominantly dependent on TLR4 (Fig. 4C).

To investigate the influence of TLR2 and TLR4 adaptors MyD88, TRIF, and TIRAP, we isolated BMDCs from MyD88−/− and TRIF−/− mice. Additionally, TIRAP blockade in DCs was achieved using a TIRAP inhibitory peptide. The effect of biglycan on cross-priming was diminished in both MyD88−/− and TRIF−/− DCs. Interestingly, although TIRAP inhibition in WT DCs succeeded in abrogating the effect of LPS, it was not sufficient to block the biglycan-induced cross-priming. TIRAP blockade in MyD88−/− and TRIF−/− DCs resulted in a strong suppression of cross-priming for both biglycan and LPS (Fig. 4D).

Biglycan modulates cross-priming via the TLR2 and TLR4 pathways involving both MyD88 and TRIF adaptors and triggers MHC II-mediated Ag presentation preferentially via the TLR4 pathway.

Biglycan triggers in vivo EAP and exacerbates myocardial T cell infiltration after myocardial infarction.

To show the distribution of biglycan within normal and inflamed cardiac tissue, we performed immunolabeling of corresponding heart sections. As expected, biglycan showed a predominantly perivascular and interstitial distribution within normal (placebo-treated) hearts, as well as a strong periileukocytic expression in EAP hearts (Fig. 5). To investigate the potential effect of biglycan on Ag-specific T cell activation in vivo with consecutive inflammation, we used two independent experimental heart injury models. In an EAP model in BALB/c mice (Fig. 6), we immunized this susceptible mouse strain with purified cardiac MyHC-α or TnI peptides, in combination with biglycan or LPS (Fig. 6A, 6B), to address a potential role for biglycan in the pathogenesis of myocarditis and pericarditis induced by autoimmune mechanisms. Expression of CD3 and CD4 was detected in heart sections. There was no significant difference in the intensity of CD3+ cell infiltration between the groups injected with biglycan- or LPS-treated DCs (Fig. 6C). Control animals (Fig. 6D) did not show any significant inflammatory activity. Within this model, we also included TLR4-deficient mice, both as donors and acceptors of biglycan-stimulated DCs. In the case of BMDCs from WT mice administered to WT mice, the pattern and intensity of biglycan-induced myocarditis were fully comparable to that induced by LPS. TLR4−/− deficient BMDCs, injected into either WT or TLR4−/− mice, were not able to trigger cardiac inflammation. In contrast, biglycan-stimulated DCs from WT mice were fully capable of inducing perimyocarditis in TLR4−/− mice. Together, these data indicated that biglycan–TLR4 signaling plays an important role at the stage of Ag processing within a DC, yet it has no significant influence on consecutive priming of T cells (Fig. 7).

In a second approach, we induced myocardial infarction by ligation of the left anterior descending coronary artery in WT (C57BL/6) and biglycan-deficient mice to assess the influence of biglycan on T cell infiltration in infarction-affected myocardium. A significantly reduced T cell infiltration was seen in biglycan knockout mice 2 wk after induction of infarction compared with controls (Fig. 8). Myocardial infarction area and infiltration of professional Ag-presenting (HR3+) cells did not significantly differ between the two groups, indicating that diminished T cell infiltration in biglycan-deficient mice does not result from reduced tissue damage or decreased monocyte infiltration, but rather from a decrease in DC–T cell interaction.

**Discussion**

In vivo mice and rat myocardial infarction models have shown strong overexpression of biglycan mRNA and protein in infarction-affected areas (33, 70). Clinical studies reported the occurrence of postmyocardial infarction pericarditis in a relevant proportion of patients (71–78). However, molecular pathomechanisms of the infarction-related myocarditis syndrome remained unclear. It is likely that DCs and infiltrating T cells play an important role. DCs have the capacity to regulate T cell responses, leading to either tolerance or immunity (79–81). Current data support the view that the overall DC-activation state, rather than surface receptor phenotype only, orchestrates the final “immunity-or-tolerance” decision (82, 83). We showed in this study that biglycan may act as an endogenous TLR2 and TLR4 ligand in tissue injury to activate...
BMDCs and to potently trigger T cell priming, leading to an enhancement of the immune response.

It is known that TLR ligands trigger signal transduction that requires recruitment of specific adaptor proteins. These adaptors associate with the cytoplasmatic domains of TLRs through specific interactions between Toll/IL-1 receptor domains of TLR and the corresponding adaptor. All TLR family members recruit the MyD88 adaptor, with the exception of TLR3, which exclusively uses TRIF (84). Our in vitro assays showed that the biglycan-mediated boost of cross-presentation requires TLR2 and TLR4 and involves MyD88, TRIF, and TIRAP adaptors. In experiments with TLR2\(^{-/-}\), TLR4\(^{-/-}\), MyD88\(^{-/-}\), and TRIF\(^{-/-}\) DCs, biglycan apparently acted through both receptors, with corresponding adaptors to enhance T cell priming; the absence of either TLR2 or TLR4, as well as the absence of TRIF (involved in TLR4, but not TLR2 signaling), led to almost complete inhibition of the effect. A similar outcome was achieved through a synchronous blockade of both the TLR2 and TLR4 pathways (MyD88\(^{-/-}\); TIRAP blockade). Therefore, our data support the assumption that biglycan-induced priming of T cells may require coaction of the TLR2 and TLR4 pathways (28, 37, 85–87). This observation is in line with our previous study showing that biglycan acts as a strong autocrine and paracrine activator of both TLR2 and TLR4 signaling in Mφs (37). This agrees with published data on the formation of heterodimers between TLR2 and other TLRs (including TLR4) (88, 89). EAP (also referred to in the literature as experimental autoimmune myocarditis) is a model originally applied to mimic postinfectious myocarditis. EAP can be induced in susceptible mouse strains (BALB/c, A/J) by immunizing with self-peptides derived from MyHC-\(\alpha\) or TnI, together with a strong adjuvant, or by injecting peptide-loaded DCs (62, 67, 90). It was demonstrated that EAP is a T cell-mediated autoimmune disease that requires a major effector role for CD4\(^{+}\) T cells and an initiating role for CD8\(^{+}\) T cells (67, 91). In other organ-specific autoimmune models, such as experimental autoimmune encephalitis and multiple sclerosis, it was shown that CD8\(^{+}\) T cells may also be important.
in the chronic phase of the disease (92, 93). Collectively, these findings indicated that immune tolerance can be broken by damage during infection, resulting in the release of self-Ags and the activation of DCs, followed by the priming of autoreactive T cells. Biglycan is ubiquitously distributed in the heart (94). We have now shown that biglycan may induce experimental autoimmune periarditis and myocarditis via potent TLR4-mediated stimulation of specific cardiac peptide presentation, enabling an autoimmune response to develop without infection. Via induction of myocardial infarction in WT and biglycan-deficient mice, we showed that biglycan can play an important role in T cell activation in infection-affected cardiac tissue, without influencing postinfarction fibrosis or infiltration of APCs. Based on our data, we propose that biglycan acts in myocardial lesions as a potent amplifier of specific cardiomyocyte Ag (TnI, MyHC-α) presentation by infiltrating DCs and Mφs to successfully prime T cells and cause an immune autoreactive response. Hence, to our knowledge, our study is the first line of evidence to identify the extracellular matrix protein biglycan as a potential link between peptide Ag presentation and TLR pathways, as well as an endogenous enhancer of autoimmune response in damaged heart tissue.

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Disclosures

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References


