Published in final edited form as:
J Mol Cell Cardiol. 2010 March ; 48(3): 538–543. doi:10.1016/j.yjmcc.2009.06.015.

Osteopontin: role in extracellular matrix deposition and myocardial remodeling post-MI

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Abstract

Remodeling after myocardial infarction (MI) associates with left ventricular (LV) dilation, decreased cardiac function and increased mortality. The dynamic synthesis and breakdown of extracellular matrix (ECM) proteins play a significant role in myocardial remodeling post-MI. Expression of osteopontin (OPN) increases in the heart post-MI. Evidence has been provided that lack of OPN induces LV dilation which associates with decreased collagen synthesis and deposition. Inhibition of matrix metalloproteinases, key players in ECM remodeling process post-MI, increased ECM deposition (fibrosis) and improved LV function in mice lacking OPN after MI. This review summarizes - 1) signaling pathways leading to increased expression of OPN in the heart; 2) the alterations in the structure and function of the heart post-MI in mice lacking OPN; and 3) mechanisms involved in OPN-mediated ECM remodeling post-MI.

Keywords

Osteopontin; ECM; MMPs; myocardial infarction; myocardial remodeling

1. Introduction

Cardiac chambers have the capacity to remodel their size and configuration in response to chronic changes in hemodynamic load. The changes in chamber volume and mass occur normally during development to adulthood. Myocardial remodeling in response to hemodynamic overload, e.g. after a myocardial infarction (MI), induces complex architectural changes involving the infarcted and non-infarcted myocardium leading to chamber enlargement, infarct thinning (also called infarct expansion) and dysfunction [1;2]. Patients exhibiting extensive infarct expansion after MI are more likely to experience complications such as development of congestive heart failure, aneurysm formation and myocardial rupture.

A large body of literature now supports a central role for extracellular matrix (ECM) proteins in the regulation of numerous cellular functions [3-5]. The structure and composition of ECM changes significantly within all regions of the LV post-MI: the MI region, the viable myocardium within the border zone, and the remote region. ECM remodeling occurs throughout the LV myocardium in a time- and region-dependent manner which in turn has the

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potential to affect the overall geometry and function of the LV [5]. The components of ECM include basic structural proteins such as collagen and elastin, and specialized proteins such as fibronectin, proteoglycans and matricellular proteins. Matricellular proteins are a class of non-structural ECM proteins exerting regulatory functions, most likely through their interactions with cell surface receptors, the structural proteins, and soluble extracellular factors such as growth factors and cytokines. Some of these proteins are members of the SIBLINGs (small-integrin binding ligand N-linked glycoproteins) family and act through integrin receptors [6; 7]. The matricellular protein family includes osteopontin (OPN), tenascin-C, tenascin-X, osteonectin, thrombospondin-1 and thrombospondin-2. The expression of matricellular proteins is almost absent during postnatal life. However, the expression reappears during tumor growth and after tissue injury. This review will focus on OPN expression in the heart, and will discuss its role in myocardial remodeling post-MI.

2. Osteopontin: a protein with multiple functions

OPN, also called cytokine Eta-1, is synthesized in a variety of tissues and cells and secreted into body fluids. Full-length human OPN protein consists of 314 amino acid residues with a predicted molecular mass of ~32 kDa. Due to extensive post-translational modifications and negative charge resulting from the presence of acidic amino acids, apparent molecular weight of OPN can range from 45 to 75 kDa on SDS-PAGE. The functional domains of OPN are well conserved among species [8]. The central integrin binding motif RGD (Arg-Gly-Asp) is completely conserved. A high degree of conservation also exists in the neighboring thrombin cleavage site and cryptic integrin attachment motif SVVYGLR (Ser-Val-Val-Tyr-Gly-Leu-Arg), which becomes accessible upon cleavage of OPN by thrombin. The mineral binding poly-aspartate region is also conserved, although the overall number of consecutive aspartic acid residues varies. Many of the phosphorylated and glycosylated sites are well conserved. OPN interacts with αvβ1, αvβ3 and αvβ5 integrins in an RGD-dependent manner [8]. Evidence has been provided for the interaction of OPN with α5β1 and α9β1 integrins in a non-RGD-dependent manner.

OPN, a multifunctional protein, is suggested to play a significant role in a variety of biological processes, including bone resorption, immune cell activation, inhibition of vascular calcification and ECM remodeling [8-12]. Post-translational modifications of OPN can impact biological functions of OPN [8]. For example, highly phosphorylated milk OPN stimulates *in vitro* bone resorption to a greater extent than unphosphorylated recombinant OPN. Phosphorylated OPN forms a heat stable complex with fibronectin in normal rat kidney cells, suggesting that phosphorylated form of OPN is an integral component of ECM. Native phosphorylated OPN inhibits calcification of human smooth muscle cells in culture, whereas unphosphorylated or enzymatically dephosphorylated OPN has no effect. Reduction in sialic acid content (O-liked glycosylation) decreases receptor-mediated localization of OPN to the surface of transformed cells. Furthermore, proteolytic cleavage of OPN by thrombin and MMPs enhances its adhesion properties as compared to the full-length protein [12]. OPN is proposed as a key cytokine involved in immune cell recruitment and type-1 (Th1) cytokine expression at sites of inflammation [11;13]. With respect to cardiovascular diseases, OPN is suggested to play a crucial role in atherosclerosis, valvular stenosis, hypertrophy, MI and heart failure [10;12].

3. OPN expression in the heart

3.1. OPN Expression in the heart in models of myocardial remodeling

Under basal conditions, heart expresses only low levels of OPN [14;15]. However, OPN expression increases markedly in the heart under several pathological states [15-21]. Increased expression of OPN is shown to be associated with the development of heart failure [18]. OPN

expression increases in infarct as well as non-infarct regions of the heart post-MI [15]. At day 3 post-MI, OPN mRNA levels were increased by 37- to 40-fold in the infarct region. OPN mRNA levels started to decline from its peak 7 days post-MI, but remained increased above the sham levels 14 and 28 days post-MI. In the non-infarct LV, OPN expression was biphasic, with peaks at 3 and 28 days post-MI [15]. In situ hybridization of heart sections 7 days post-MI demonstrated abundant expression of OPN mRNA in the area of infarction. The expression of OPN in the infarct region was primarily localized to nonmuscle and infiltrating cells. Diffuse OPN message was also detectable in the non-infarct LV, with more focal message associated with blood vessels, possibly in endothelial and/or smooth muscle cells. Immonohistochemical analysis demonstrated positive staining for OPN protein mainly in the interstitium in the infarct and non-infarct LV regions 28 days post-MI [15]. In situ hybridization of the heart sections obtained from spontaneously hypertensive rats with heart failure revealed abundant expression of OPN mRNA, primarily in non-myocytes (possibly infiltrating macrophages and fibroblasts) in the interstitial and perivascular space [18]. Increased OPN expression was observed in the interstitium (mainly infiltrating macrophages) in myocardium of rats with thermal injury and Syrian hamsters with heritable cardiomyopathy [16;22]. Infiltrating macrophages were detected as main source of OPN in chronic myocarditis [21]. LV hypertrophy associates with increased OPN expression in the heart [17;23]. However, increased OPN expression was mainly observed in cardiac myocytes [17]. Increased OPN protein levels, mainly associated with cardiac myocytes, were also observed during streptozotocin-induced diabetic cardiomyopathy [20]. Collectively, these observations suggest infiltrating macrophages as the main source of OPN. However, other resident cell-types of the heart are capable of expressing OPN under different pathologies.

3.2. OPN in patients with MI and heart failure

Immunohistochemical analysis of myocardial biopsies obtained from patients with heart failure due to dilated cardiomyopathy (DCM) demonstrated increased OPN expression in cardiac myocytes [24]. This increased OPN expression in cardiac myocytes correlated significantly with impaired LV function assessed by hemodynamic data (LV ejection fraction, $R = -0.828$; RV ejection fraction, $R = -0.671$; LV end systolic volume index, $R = 0.751$; LV end diastolic index, $R=0.685$; LV end diastolic pressure, $R = 0.461$; all $P<0.05$). In situ hybridization showed cardiac myocytes as the major source of OPN message in patients with DCM [25]. Here, OPN mRNA levels correlated positively with collagen type I levels ($r = 0.60$; $P<0.01$) and collagen volume fraction ($r = 0.52$; $P<0.001$), but correlated negatively with LV ejection fraction ($r = -0.43$; $P<0.01$). A limitation of the above study was the exclusion of patients with ischemic heart disease. A time-dependent analysis of plasma OPN levels in patients who underwent successful reperfusion within 12 h after the onset of anterior-wall acute MI showed that plasma OPN levels began to increase on day 2 and reached a maximal level on days 3 through 5 [26]. The OPN levels were still increased on day 7 but returned gradually to the normal range by day 14. The area under the curve for plasma OPN levels for 14 days after acute MI was significantly correlated with LV end-systolic volume index ($r = 0.66$; $P<0.01$), LV end-diastolic volume index ($r = 0.50$; $P<0.05$) and LV ejection fraction ($r = -0.55$; $P<0.05$). Tamura and colleagues [27] measured OPN released from the heart into coronary circulation in patients with a previous (for >3 months) anterior wall MI. Blood samples were obtained from the aortic root and coronary sinus with the intent that the difference between the plasma concentrations of OPN in the aortic root and coronary sinus will reflect the cardiac production of OPN released into the coronary circulation. It was noted that the plasma OPN concentrations were significantly higher in the coronary sinus than in the aortic root (672±446 vs 610±398 ng/ml; $p=0.02$) indicating that OPN is released from the heart into the coronary circulation at the chronic MI stage. The transcardiac gradient of plasma OPN concentration correlated negatively with LV ejection fraction ($r = -0.55$; $p=0.0005$) and positively with LV end-diastolic ($r = 0.63$,

p=0.0001) and end-systolic volume (r = 0.79, p<0.0001) indexes. These studies provide evidence that increased OPN expression associates with post-MI LV remodeling in humans.

3.3. *In vitro* OPN expression in different cell-types of the heart

Cardiac myocytes occupy approximately 75% of normal heart tissue volume. However, myocytes only account for 30−40% of cell numbers. The majority of the remaining cells are non-myocytes which include fibroblasts, endothelial cells and vascular smooth muscle cells. Northern blot analysis showed that fresh primary isolates of adult rat ventricular myocytes exhibit low basal expression of OPN mRNA [14]. On the other hand, OPN mRNA was readily detectable in confluent primary cultures of cardiac microvascular endothelial cells (CMECs) and cardiac fibroblasts (ARCFs) isolated from adult rat heart [14;28]. Rat aortic smooth cells are also shown to express OPN [29].

3.4. Stimuli involved in increased OPN expression

Expression of OPN appears to be differentially regulated in different cell-types of the heart in response to different stimulants. Glucocorticoids increase OPN expression in adult cardiac myocytes and CMECs. A combination of cytokines, interleukin-1β (IL-1β) and interferon-γ (IFN-γ) increased OPN expression in CMECs, not in cardiac myocytes [14]. Angiotensin II (Ang II) increases OPN gene expression in ARCFs and CMECs, but not in cardiac myocytes [28;30]. IL-1β, IFN-γ and tumor necrosis factor-α (TNF-α) alone failed to increase OPN expression in ARCFs. However, a combination of Ang II with IL-1β or TNF-α, not IFN-γ, increased OPN mRNA more than Ang II alone. This increase in OPN expression in the presence of cytokines occurred via nitric oxide independent mechanism [28]. Importance of Ang II in the increased OPN expression is corroborated by the observations that angiotensin converting enzyme inhibitor, captopril, inhibits expression of OPN in the myocardium of spontaneously hypertensive rats with heart failure [18], and blockades of Ang II and aldosterone reduce OPN expression in the heart post-MI [31].

The differential OPN gene expression in response to different stimuli could be due to the activation of different signaling pathways. However, activation of ERK1/2 (a member of mitogen activated protein kinase, MAPK, family) and production of reactive oxygen species appears to be a common mechanism involved in the increased OPN expression in CMECs and ARCFs in response to Ang II. In CMECs, ANG II elicited robust phosphorylation of p42/44 MAPK and increased superoxide production. Both of these effects were blocked by diphenylene iodonium (DPI), an inhibitor of the flavoprotein component of NAD(P)H oxidase. PD98059, an inhibitor of p42/44 MAPK pathway, and DPI each inhibited ANG II-stimulated increases in OPN expression. These data suggest that ERK1/2 is a critical component of the ROS-sensitive signaling pathways activated by ANG II and plays a key role in the regulation of OPN gene expression in CMECs [30]. Similar observations were made in ARCFs in response to Ang II [28]. The involvement of ERK1/2 in OPN expression is also highlighted by a recent observation where chronic myocarditis associated with activated ERK1/2 and increased OPN expression in the mouse myocardium. Treatment of mice with vitamin D analog, ZK191784, decreased activation of ERK1/2 as well as OPN expression [21]. In ARCFs, we analyzed the involvement of other members of MAPK family, such as JNKs and p38 kinase [28]. It was observed that Ang II alone or in combination with IL-1β activates JNKs, not p38 kinase. Inhibition of JNKs only partially inhibits Ang II+IL-1β-mediated increase in OPN expression, while a combined inhibition of ERK1/2 and JNKs almost completely inhibits Ang II+IL-1β-mediated increases in OPN expression [28]. Taken together, these studies suggest that Ang II and cytokines play a significant role in the increased OPN expression in interstitial cells and in the heart post-MI. However, stimulus for increased OPN expression in cardiac myocytes during cardiac hypertrophy and diabetic cardiomyopathy remains to be determined.

4. OPN in myocardial remodeling post-MI

4.1. Physiological significance of increased OPN expression post-MI

Increased OPN expression in the heart post-MI suggests a role for OPN in myocardial remodeling. Genetic evidence for the role of OPN in myocardial remodeling was obtained using mice lacking OPN (OPN−/−) and MI as a model of myocardial remodeling. There was no difference in the post-MI mortality rates (48 h or 28 days post-MI) between wild-type (WT) and OPN−/− mice. The heart weight/body weight ratio and septal wall thickness increased to a similar degree in WT and OPN−/− mice post-MI. MI size, measured 28 days post-MI as a percentage of the LV circumference, was not different between WT and OPN−/− groups [15]. However, OPN appeared necessary for maintaining the heart function post-MI since mice lacking OPN exhibited exaggerated LV dilation. Measurement of LV end-diastolic pressure-volume relationship using Langendorff perfusion analysis demonstrated increased LV chamber volume (LV dilation) in WT mice 28 days post-MI (versus sham). Interestingly, the increase in LV chamber volume in OPN−/− mice after MI was twice as much as in WT-MI mice [15]. Echocardiographic analyses of the heart 14 days post-MI indicated increased LV end systolic and diastolic diameters in WT mice (versus sham). However, the increase in LV end systolic and diastolic diameters was significantly higher in OPN−/− mice ($P<0.05$ vs WT-MI) [32]. Thus, increased OPN expression protects the heart against LV dilation and plays a beneficial role in post-MI LV remodeling.

4.2. Reduced fibrosis in mice lacking OPN

LV chamber enlargement post-MI occurs due to infarct expansion and dilatation of the non-infarct LV. No change in infarct size between WT and OPN−/− hearts post-MI suggests that chamber enlargement is most likely due to dilatation of non-infarct LV, which can occur due to a number of mechanisms including cardiac myocyte apoptosis [33] and/or side-to-side slippage of the myocytes in the absence of increased fibrosis [34]. The number of apoptotic myocytes was not different in the myocardium of WT and OPN−/− mice 28 days post-MI [15]. Therefore, increased cardiac myocyte apoptosis may not be the main mechanism leading to greater post-MI LV dilatation in OPN−/− mice. Of note, lack of OPN associates with reduced cardiac cell/myocyte apoptosis in the myocardium of OPN−/− mice in other models of myocardial remodeling [19;20]. These differential effects of OPN on cardiac myocyte apoptosis under different pathologies may include different neurohormonal response under diverse pathological conditions, cell-type/s involved in the expression of OPN, receptor/s (integrins or CD44) stimulated by OPN and/or differential post-translational modifications of OPN, including phosphorylation and glycosylation.

A well-organized ECM plays an important role in maintaining the strength and organization of the cardiac tissue. Analysis of collagen weave (fibrosis) using scanning electron microscopy indicated presence of normal collagen content and fiber size in the myocardium of WT-sham group [15]. The fibrillar collagen weave appeared disrupted in the myocardium of sham OPN −/− mice. Interestingly, total fibrillar collagen and the size and frequency of large collagen fibers (struts) was increased in the non-infarct LV of WT heart 28 days post-MI (Figure 1). However, fibrillar collagen appeared reduced in the non-infarct LV of OPN−/− heart when compared with WT-MI heart. Specifically, there was a marked decrease in thin collagen filaments (weave) between cells, as well as a lack of the larger collagen fibers seen in the WT-MI mice [15]. Immunohistochemical staining 28 days post-MI demonstrated increases in collagen I content in the infarct as well as non-infarct LV regions of WT, not in OPN−/−, group. Northern analysis demonstrated no significant increase in collagen I(α1) mRNA expression in the myocardium of OPN−/− group after MI. However, collagen I(α1) mRNA was increased 3-fold in the non-infarct region of WT-MI hearts [15]. These observations led to the conclusion that increased OPN expression in the heart post-MI plays a crucial role in regulating the post-

MI LV remodeling, at least in part, by promoting collagen synthesis and accumulation [15]. It is interesting to note that lack of OPN also associates with reduced fibrosis in other models of myocardial remodeling, including aldosterone infusion, streptozotocin-induced diabetic cardiomyopathy and Ang-II infusion [19;20;35].

4.3. Role of OPN in the regulation of MMPs

Matrix metalloproteinases (MMPs), a family of endopeptidases, have the ability to degrade extracellular matrix proteins, and therefore play a fundamental role in tissue remodeling. MMPs are implicated in the remodeling processes of the heart during chronic heart failure and following MI [4;5]. Therefore, OPN-mediated inhibition of expression and activity of MMPs may represent another possibility leading to enhanced collagen deposition in WT-MI hearts. This possibility was explored using ARCFs and purified OPN protein. It was demonstrated that OPN inhibits IL-1β-stimulated increases in expression and activity of MMP-2 and MMP-9 [36]. Figure 2 demonstrates that OPN alone has no effect on MMP activity. However, OPN in the presence of IL-1β inhibits MMP-2 and MMP-9 activities in a dose dependent manner. Other data provided evidence that OPN, acting via β3 integrins, inhibits IL-1β-stimulated increases in MMP-2 and MMP-9 activity, at least in part, via the involvement of PKC-ζ [36]. Expression of IL-1β increases in the heart post-MI 37. Therefore, OPN may enhance collagen deposition in the heart post-MI by inhibiting IL-1β-stimulated increases in MMP expression and activity.

4.4. LV dilation and inhibition of MMPs in mice lacking OPN

The role of MMP inhibition in mice lacking OPN was investigated using PD166793, a broad spectrum inhibitor of MMPs [38]. M-mode echocardiography 14 days post-MI indicated that MMP inhibition decreases post-MI LV dilation in OPN−/− mice, not in WT [32]. Quantitative analysis of fibrosis using trichrome-stained sections indicated increased fibrosis in both WT and OPN−/− mice 14 days post-MI as compared to their respective shams. However, the increase in post-MI fibrosis was significantly lower in OPN−/− mice when compared to WT group. PD166793 treatment significantly increased fibrosis only in OPN−/− mice 14 days post-MI (percent fibrosis; MI, 9.8±0.99; MI+PD, 18.39±2.39; $P<0.01$ vs MI). At 3 days post-MI, MMP-2 activity was higher in the non-infarct LV of OPN−/− mice which was inhibited by PD166793 [32]. Activation of MMPs, specifically MMP-2, is suggested to decrease cardiac tissue tensile strength and cause systolic and diastolic dysfunction [39]. Cardiac-specific expression of MMP-2 induces development of cardiac contractile dysfunction in the absence of superimposed injury [40]. On the other hand, targeted deletion of MMP-2 attenuates early cardiac rupture and the development of subsequent LV dysfunction post-MI [41]. The observation that MMP inhibition decreases MMP-2 activity and enhances the fibrotic response in mice lacking OPN [32] raises an interesting possibility that interplay between OPN and MMP-2 may have a significant role in heart function post-MI. However, further studies using specific inhibition of MMP-2 or transgenic approaches are needed to prove this thesis. Of note, PD166793 inhibits other members of the MMP superfamily, including MMP-3, -13, -1, -7 and 14. Evidence has been provided that PD166793 also has the potential to inhibit AMP deaminase activity [38].

4.5. Other functions of OPN related to ECM remodeling

OPN is shown to interact with fibronectin and collagen types I, II, III, IV and V [42;43]. Using cardiac fibroblasts isolated from the myocardium of WT and OPN−/− mice, OPN is demonstrated to be an important factor modulating cardiac fibroblast growth, adhesion to extracellular matrix proteins including collagen I, fibronectin, laminin and vitronectin and collagen gel contraction [44;45]. Cardiac OPN−/− fibroblasts exhibit less spreading, less resistance to detachment by shear stress and reduction in collagen gel contraction as compared to cardiac fibroblasts isolated from the myocardium of WT mice. These defects in OPN−/−

fibroblasts were partially restored by ectopic expression of OPN [46]. Of note, OPN is shown to be a substrate for cleavage by MMP-3 and MMP-7. OPN activity as an adhesion molecule and migratory stimulus is enhanced following cleavage by MMPs [47]. OPN is required for myofibroblast differentiation induced by transforming growth factor-β1 [46]. Myofibroblasts help maintain the integrity of the damaged tissue by contracting the newly deposited ECM, thereby promoting wound closure [48]. OPN acts as a chemo-attractant for vascular endothelial and smooth muscle cells and is shown to promote vascular cell adhesion and spreading [29; 49]. We have shown that lack of OPN results in impairment of myocardial capillary and arteriolar development post-MI [50]. Absence of OPN significantly reduced angiogenesis both *in vivo* and *in vitro*. The reduced angiogenic response in CMECs isolated from the myocardium of OPN−/− mice was restored by treatment with purified OPN protein. Evidence has been provided that OPN inhibits cytokine-inducible isoform of nitric oxide synthase in adult cardiac myocytes and CMECs [14].

5. Conclusion

It is clear that OPN expression increases in the heart after MI. Increased expression of OPN has the potential to increase cardiac fibrosis and protect the heart against LV dilation after MI by various mechanisms as outlined in Figure 3. OPN-mediated myofibroblast differentiation, increased collagen I expression and decreased MMP expression and activity may ultimately help improve the tensile strength of the infarct scar. Other functions of OPN, such as modulation of cardiac fibroblast growth, adhesion and spreading, may also have significant role in myocardial remodeling post-MI by maintaining the cell mass at the site of injury.

Despite these advances in understanding the biological significance of OPN in the heart post-MI, several questions remain to be explored - How does collagen quality change post-MI in the presence and absence of OPN? Is OPN-mediated increase in fibrosis indeed beneficial to the heart long-term, i.e. >28 days post-MI? Whether specific inhibition or deletion of MMP-2 in OPN−/− mice can inhibit the LV dilation post-MI? Whether overexpression of OPN, specifically in the heart, can provide greater benefit to the heart post-MI? What is the role of proteolytically cleaved fragments of OPN in the heart? What are the stimuli for increased OPN expression in cardiac myocytes during cardiac hypertrophy and diabetic cardiomyopathy? Is OPN synthesized by cardiac myocytes is different, with respect to post-translational modifications, from the OPN synthesized by other cell-types of the heart, e.g., fibroblasts, endothelial cells, macrophages etc? How increased expression of OPN in cardiac myocytes during cardiac hypertrophy and diabetic cardiomyopathy mediates cardiac myocyte apoptosis? Future studies aimed at identifying the stimuli involved in increased expression of OPN in the heart and understanding the mechanism by which increased OPN expression orchestrates the fibrotic and repair response may be warranted to define the therapeutic potential of OPN in the remodeling processes of the heart post-MI.

Acknowledgments

This work is supported by National Institutes of Health (Grant numbers HL-091405 and HL-092459) and a Merit Review Grant from the Department of Veterans Affairs.

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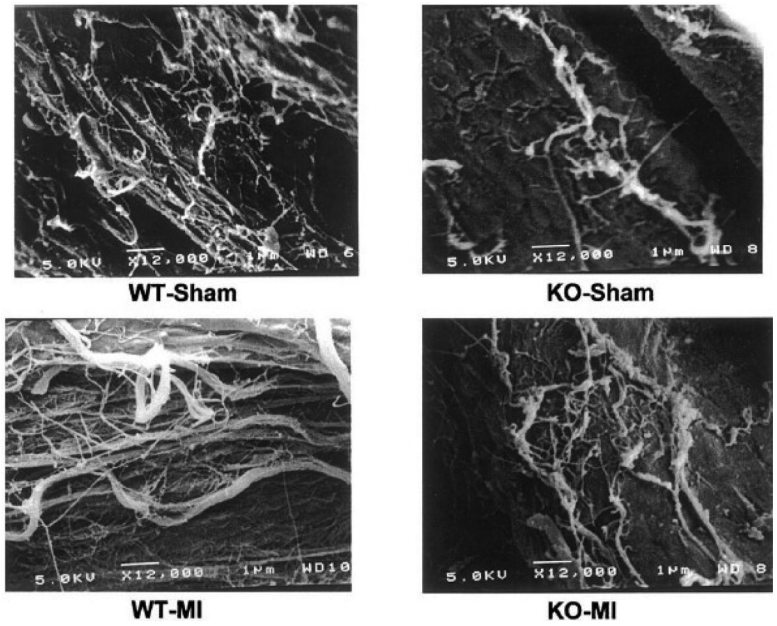

Figure 1.
SEM analysis of non-infarct LV 28 days post-MI. WT-sham heart showed normal collagen content and fiber size (top left), whereas WT-MI heart showed increased thin collagen filaments and numerous larger collagen fibers (bottom left). The fibrillar collagen weave appeared reduced or disrupted in the KO group (top right and bottom right). WT, wild-type; KO, OPN knockout; MI, myocardial infarction [15].

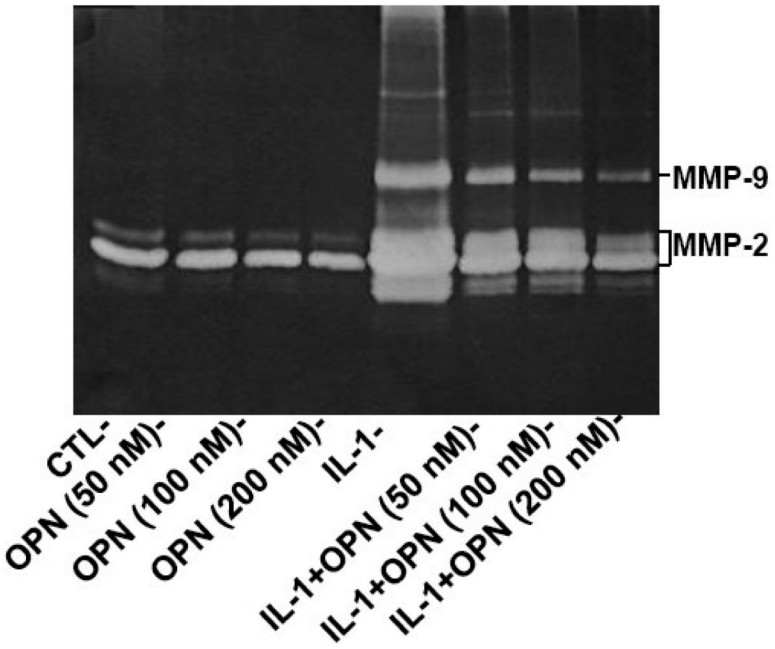

Figure 2. OPN inhibits IL-1β-stimulated MMP activity

Confluent cultures of cardiac fibroblasts were pretreated with OPN (50 − 200 nM) for 30 min followed by treatment with IL-1β (4 ng/ml) for 48 h. MMP activity in conditioned media was measured using in-gel zymography [36].

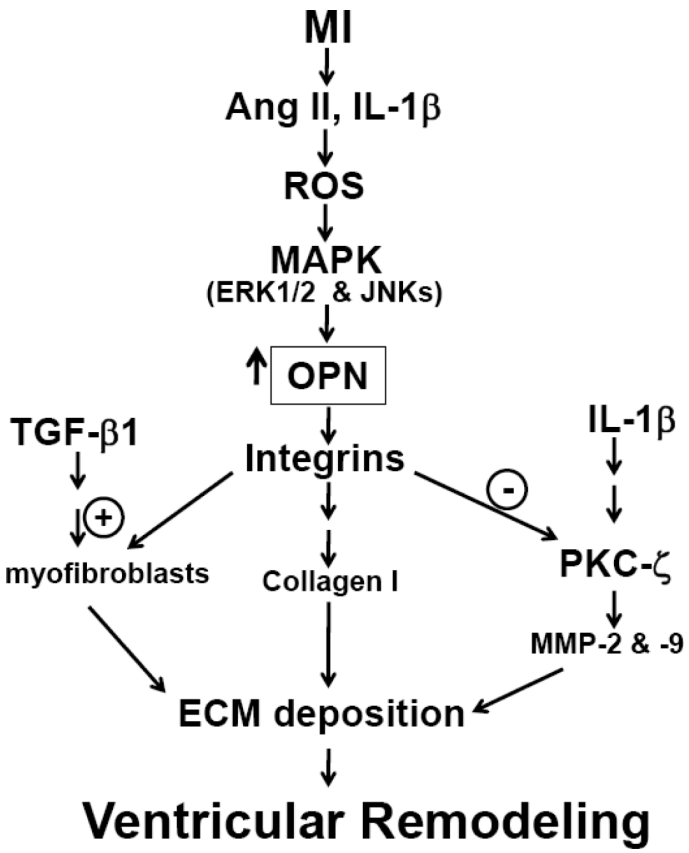

Figure 3.
Summary diagram illustrating increased OPN expression in cardiac cells and mechanism/s by which OPN may play a role in myocardial fibrosis and remodeling post-MI.