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Review article

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.

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Contents

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

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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 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 $\alpha v\beta 1$, $\alpha v\beta 3$ and $\alpha v\beta 5$ integrins in an RGD-dependent manner [8]. Evidence has been provided for the interaction of OPN with $\alpha 5\beta 1$ and $\alpha 9\beta 1$ 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 et al. [27] measured OPN released from