Review

MOLECULAR METABOLISM

Osteopontin: A novel regulator at the cross roads of inflammation, obesity and diabetes

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ABSTRACT

Since its first description more than 20 years ago osteopontin has emerged as an active player in many physiological and pathological processes, including biomineralization, tissue remodeling and inflammation. As an extracellular matrix protein and proinflammatory cytokine osteopontin is thought to facilitate the recruitment of monocytes/macrophages and to mediate cytokine secretion in leukocytes. Modulation of immune cell response by osteopontin has been associated with various inflammatory diseases and may play a pivotal role in the development of adipose tissue inflammation and insulin resistance. Here we summarize recent findings on the role of osteopontin in metabolic disorders, particularly focusing on diabetes and obesity.

Keywords Osteopontin; Biomineralization; Tissue remodeling; Inflammation; Obesity; Insulin resistance; Insulin sensitivity; Non-alcoholic fatty liver disease; Diabetic nephropathy

1. INTRODUCTION

Osteopontin (OPN), also known as secreted phosphoprotein 1 (SPP 1), 44 kDa bone phosphoprotein, sialoprotein 1, 2ar, uropontin, and early T-lymphocyte activation-1 (Eta-1) is a secreted matricellular protein that was first identified in 1985 by Heingard et al. as sialoprotein derived from bovine bone matrix [1]. The most commonly used name osteopontin is derived from "osteon", the Greek word for bone, and "pons", the Latin word for bridge illustrating its function as a linking protein and crucial factor in bone homeostasis [2].

OPN is a negatively charged aspartic acid-rich, N-linked glycosylated phosphoprotein composed of 314 amino acid residues [3–5]. The human gene for OPN has been localized on the long arm of chromosome 4q13 directly related to four similar genes encoding for bone sialoprotein (BSP), dentin matrix protein 1 (DMP1), dentin sialophosphoprotein (DSPP) and matrix extracellular phosphoglycoprotein (MEPE) [6,7]. Due to common functional motifs and domains these five integrin-binding glycophosphoproteins are categorized as the so called SIBLING proteins (small integrin-binding ligand N-linked glycoproteins) [8]. OPN is encoded by a single copy gene, but exists in various isoforms as a result of alternative splicing, alternative translation and different posttranslational modifications (PTMs), which allow for a molecular weight ranging from 41 to 75 kDa [9–13]. To date three splice variants of the human OPN transcript have been identified: OPN a, the full-length isoform; OPN b which lacks exon 5 and OPN c which lacks exon 4 [11]. OPN has primarily been described as a secreted protein involved in several physiological as well as pathological events. However, current evidence suggests that OPN can also be found in the cytoplasm and the nucleus [14]. This form of intracellular OPN (iOPN) is the result of alternative translation and has biological functions distinct from those of secreted OPN (sOPN) [15].

OPN is expressed in various cell types and tissues including preosteoblasts, osteoblasts, osteocytes [16], chondrocytes [17], fibroblasts [18], dendritic cells, macrophages and T-cells [19], hepatocytes [20], smooth muscle cells [21], skeletal muscle [22], endothelial cells [23], inner ear [24], brain [25], placenta and mammary glands [26], deciduum and kidney [5,27]. Extracellular OPN functions through its interactions with multiple cell surface receptors, including various integrins ($\alpha v\beta 1$, $\alpha v\beta 3$, $\alpha v\beta 5$, $\alpha v\beta 6$, $\alpha 4\beta 1$, $\alpha 5\beta 1$, $\alpha 8\beta 1$, and $\alpha 9\beta 1$) and CD44 [15,16] thereby regulating cellular processes such as biomineralization, tissue remodeling and immune regulation [4,28,29]. Abundant evidence suggests that OPN plays a critical role in chronic inflammatory diseases, including multiple sclerosis [30], Crohn's disease [31] and other autoimmune disorders [32,33], several types of cancer [34–36] and cardiovascular diseases [37–40]. Furthermore, OPN may play a pivotal role in the development of adipose tissue inflammation and insulin resistance [29,41]. In this review, we will summarize the current knowledge on the role of OPN in metabolic disorders, particularly focusing on diabetes and obesity.

2. OPN IN BIOMINERALIZATION

It is now well recognized that one major physiological function of OPN is the control of biomineralization. As a member of the SIBLING protein family with overall negative charge, OPN is able to directly bind to specific apatite crystal faces thereby governing its function as a mineralization inhibitor [4,42]. Hence, $OPN^{-/-}$ bones unlike those from wild type mice are hypermineralized and more fragile [4,43]. Furthermore, OPN is not only critical for bone mineralization, but is also strongly upregulated at sites of ectopic, pathologic calcification — such as vascular calcification [44], valvular calcification [45], renal crystal formation [46], and gallstone formation [47].

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3. OPN IN TISSUE REMODELING PROCESSES

Although it is not required for normal bone formation and development, OPN participates as an essential component in the bone remodeling process [48,49]. Bone cells secrete OPN physiologically during the process of bone remodeling and increase OPN expression in response to mechanical stimuli [50,51]. OPN appears to stimulate adhesion, migration and bone resorption by osteoclasts [52]. OPN function in osteoclasts involves the stimulation of CD44 expression on the osteoclast surface, which was shown to be required for osteoclast motility and bone resorption. Consistently, resorption of ectopic bone is substantially impaired in the absence of OPN [53]. OPN has also been shown to regulate remodeling of soft tissues in response to pathologic stimuli. For example, in heart failure and cardiac remodeling there appears to be a sophisticated balance of OPN expression: strongly increased levels of OPN may induce deleterious fibrosis and hypertrophy while sufficient levels of OPN are needed to prevent left ventricular (LV) dilatation [37].

4. OPN IN INFLAMMATION

Multiple studies have demonstrated that OPN is expressed by inflammatory cells such as macrophages and highly induced during inflammatory activation [19,54].

OPN appears to be constitutively expressed but in all cells studied it is rapidly upregulated following cellular activation by a variety of growth factors and cytokines (including LPS, NO, Ang II, IL-1β, IL-2, IL-3, IFN-γ, TNF-α, TGFβ) [55,56]. Until this date the molecular mechanisms that regulate OPN expression in macrophages during inflammation remain incompletely understood. The OPN promoter is remarkably responsive and contains various motifs including a purine-rich sequence, an ETS-like sequence, glucocorticoid and vitamin D response elements, and IFN-inducible elements [57,58]. In LPS-stimulated macrophages OPN expression was shown to be upregulated by activation of phosphoinositide 3-kinase (PI3K), extracellular signal-regulated kinase (ERK), and c-Jun NH_2-terminal kinase (JNK). Furthermore, chromatin immunoprecipitation (ChIP) assays revealed that activator protein 1 (AP-1) binds to the proximal AP-1 site in the OPN promoter from LPS-stimulated macrophages [59]. We could demonstrate that cytokine-induced OPN expression in macrophages depends on AP-1 binding to a CCTCATGAC cognate (AP-1 consensus element) in the proximal OPN promotor between -80 and -71. Stimulation of macrophages with LXR ligands suppressed cytokine-induced OPN expression by inhibition of c-Jun/c-Fos DNA binding activities to the proximal OPN promotor, which impairs AP-1-dependent OPN transcription [60]. Recent work provided evidence that NF-κB also plays an important role during LPS-stimulated OPN expression through binding to a cis-regulatory element (GGAATTCCC between nt − 1817 and nt − 1808) in the distal OPN promotor in macrophages. Interestingly, LPS stimulation induced chromosomal loops in the OPN promotor between the NF-κB binding site and the AP-1 binding site involving the coactivator p300. These results identified an essential mechanism to establish higher order chromatin structure to regulate LPS-induced OPN expression [61]. Work by Oyama and co-workers demonstrated that phorbol 12-myristate 13-acetate (PMA)-induced OPN expression is significantly decreased by troglitazone and other PPARγ ligands in macrophages. Further experiments showed that PPARγ inhibits the OPN promoter activity, and the PPARγ-responsive region within the OPN promoter lies between -1000 and -970 relative to the transcription start site. Site-specific mutation analysis and electrophoretic mobility shift assays indicated that a homeobox-like A/T-rich sequence between -990 and -981, which functions as a binding site for PMA-induced nuclear factors other than PPARγ, mediates the repression of OPN expression by troglitazone. Moreover, concatenated A/T-rich sequences conferred the PPARγ responsiveness on the heterologous promoter. All in all, these data suggest that PPARγ ligand inhibits OPN gene expression through the interference with the binding of nuclear factors to A/T-rich sequence in macrophages [62].

It is now well recognized that OPN controls immune cell functions including monocyte adhesion [63], migration [64], differentiation [65], and phagocytosis [4,15,66]. The induction of monocyte and macrophage chemotaxis and cellular motility as well as migration by OPN occurs via direct interaction with several different cell surface receptors [67–69]. This interaction is mostly mediated by two different binding domains. As mentioned above, OPN interacts with αvβ1, αvβ3, αvβ5, αvβ6, α8β1 and α5β1 integrins through its RGD domain, while the SLAYGLR (SVVYGLR in human OPN) domain facilitates binding to α9β1, α4β1 and α4β7 integrins. Furthermore, OPN has been identified to interact with the CD44 hyaluronic acid receptor [54]. Additionally, OPN induces the expression of matrix metalloproteinase (MMP), in particular MMP-2 and MMP-9 [39,64,70]. Since these proteinases are important in degrading matrix for migrating cells, this represents an alternative mechanism by which OPN may profoundly enhance cellular migration. In vivo, evidence for OPN regulating monocyte/macrophage recruitment to sites of inflammation was provided by studies using either blocking antibodies or genetic approaches. Neutralizing antibodies to OPN diminished intradermal macrophage infiltration in response to a chemotactic peptide [63] and monocyte migration into joints leading to an inhibition of rheumatoid arthritis [71]. Impaired leukocyte recruitment in $OPN^{-/-}$ mice has further been demonstrated in a variety of different inflammatory disease processes [39,72–76]. In all these studies, $OPN^{-/-}$ mice consistently exhibited diminished leukocyte recruitment at sites of inflammation demonstrating the pivotal role of OPN to regulate leukocyte attraction during inflammation. OPN is not only critical for macrophage recruitment, but also regulates the secretion of cytokines during cell-mediated immunity [4]. OPN itself activates the transcription factors NF-κB and AP-1 and thereby potentially modulates the expression of a variety of inflammatory genes [77]. The engagement of CD44 and αvβ3 integrin by OPN induces PI3-kinase dependent Akt phosphorylation and enhances the interaction between phosphorylated Akt and IKKα/β. OPN also enhances NF-κB activation through phosphorylation and degradation of IκBα by inducing the IKKα/β activity [78,79]. Furthermore, SFK (Src family of tyrosine kinases) kinase activity was found to be required for integrin αvβ3-mediated NF-κB activation [80]. OPN-mediated activation of AP-1 is mediated by nuclear factor-inducing kinase (NIK)—ERK (extracellular signal-related kinase) and MEKK1 (also known as mitogen-activated protein kinase kinase kinase 1 (MAP3K1)—JNK1 (also known as MAPK8)) signaling. Upon binding to αvβ3, OPN also stimulates epidermal growth factor receptor (EGFR) transactivation and ERK phosphorylation [8]. Further studies revealed that OPN regulates crosstalk between NF-κB and AP-1 by p70S6K/mTOR phosphorylation which is unidirectional towards AP-1 that in turn regulates intercellular adhesion molecule-1 (ICAM-1) expression [77].

Polarization of Th cells to the Th1 or Th2 phenotypes, a critical aspect of cell-mediated immunity, is influenced by OPN which enhances Th1 and inhibits Th2 cytokine expression [75]. OPN induces macrophages to express IL-12 and stimulates T-cells to express INF-γ and CD40 ligand, which subsequently induces IL-12 expression from monocytes [75,81]. Thus, OPN provides an important early stimulus for IL-12 production at sites of inflammation. OPN further inhibits IL-10

