Osteopontin Function and Interaction with Vascular Calcification and Atherosclerosis

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Abstract

Vascular calcification is now recognized as a marker of atherosclerotic plaque burden as well as a major contributor to loss of arterial compliance and increased pulse pressure seen with age, diabetes mellitus, and renal failure. Long bones epiphyses serve as a niche of hematopoietic and mesenchymal stem cells contributing for the bone and vascular homeostasis. Hormones and peptides affect the vascular system through activation of different stem cells and transcriptional factors. Recent studies, emphasize the strong correlation between bone matrix that is secreted during bone tissue regeneration and angiogenesis. One of the most important components of bone matrix is osteopontin (OPN) that is linked to the bone regeneration and angiogenesis. OPN serves an adhesive substrate for both vascular smooth muscle (SMCs) and endothelial cells as well a potent chemo tactic factor for SMCs. OPN is present focally in human atherosclerotic coronary and carotid artery specimens but absent in no diseased coronary arteries. OPN mRNA is present in a plaque and mainly expressed in macrophages but also in smooth muscle cell and endothelial cells. Furthermore OPN is correlated with bone turnover and remodeling and may play a role in plaque calcification. The literature reported the expression of bone morphonegic protein 2a, a potent factor for osteoblast differentiation, in human atherosclerosis suggesting that the plaque calcification is an active process. Osteoblasts regulates bone formation, its precursors come from the mesenchyma of bone marrow while osteoclasts come from hematopoietic precursors. Both osteoclasts and osteoblasts produce OPN and their activities are crucial for bone remodeling besides osteoid mineralization. Mechanistically increasing evidence suggests that phosphorylated-OPN directly associates with apatite deposits and blocks crystal growth in addition to inducing RGD-mediated mineralization of cardiovascular tissues. Taken together bone marrow

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progenitors/stem cells unbalance may contribute for the vascular calcification. A better understanding of the mechanism that link between bones and blood vessels may open a new frontier to fight atherosclerosis and enhance blood vessel repair.

1 Introduction

Identifying the process and molecular mediators of atherosclerosis plaque formation are currently subject of intense investigation. The emergent idea that stem cells are involved in cells turnover and tissue maintenance open a new area of interest for researchers. Stem cells have capacity to differentiate into different tissues and they can secrete a lot of organ specific molecules that regulates its function and endogenous repair. Deregulation in their function can directly affect the organ homeostasis.

Osteopontin (OPN) is a potent regulator of hematopoietic stem cells [1]. Interestingly, besides its expression from osteoblasts, osteoclasts and myelomonocytic cells, OPN is focally present in human atherosclerotic coronary and carotid artery specimens but absent in no coronary artery disease [2]. OPN mRNA is present in a plaque and mainly expressed in macrophages but also in smooth muscle cell and endothelial cells [3]. Furthermore OPN is correlated with bone turnover and remodeling and may play a role in plaque calcification. The literature reported the expression of bone morphonegic protein 2a, a potent factor for osteoblast differentiation, in human atherosclerosis suggesting that the plaque calcification is an active process [4]. A large number of studies have demonstrated a relationship between vascular disease and bone pathology. The coexistence of osteoporosis and features of atherosclerosis, particularly vascular calcification, has been consistently demonstrated and is most prevalent in postmenopausal women and elderly people [2-6]. These observations suggest that there are common pathways which negatively affect bone metabolism and the vasculature.

2 Stem Cell Niches

Throughout life, stem cells persist in numerous tissues replacing mature cells lost during physical activity or injury. However, the function of stem cells and other progenitors decline with age [7] consequently aging tissues demonstrate decreased repair capacity and increased preponderance for degenerative disorders [8]. Tissue resident stem cells and mesenchymal stem cells (MSCs) are ubiquitously distributed and have been characterized in bone marrow, adipose tissue, skeletal muscle, dermis, and umbilical cord [9]. Age-related decline in the quantity of MSCs in bone marrow has been reported in rodents, monkeys, and humans [10-12]. The genetic alterations responsible for aging of stem cells include changes that directly affect cell cycle, genetic stability, and DNA damage and repair [13]. Gender differences are important in coronary artery disease, as demonstrated by different manifestation of coronary atherosclerosis in premenopausal women compared with age-matched men. The protective effects of estrogen are mediated through the estrogen receptor alpha, and 17-β estradiol (E2) that stimulates migration and proliferation of endothelial cells through activation of endothelial nitric oxide synthase (eNOS), prostacyclin, endothelin, β-fibroblast growth factor (FGF), and vascular endothelial growth factor–receptor 2 (VEGF-R2) [14, 15]. Premenopausal women have

higher numbers of circulating endothelial progenitor stem cells (EPCs) compared to age-matched men. The level of circulating EPCs synchronizes with menstrual cycle and is the highest during the fertile period. OPN has a role in the homing of EPCs to the region of vascular damage [16].

OPN expression in injured vessel wall and in bone marrow-derived EPCs appears to be essential for E2-induced enhancement of re-endothelialization. Its expression is up-regulated by interleukin-1, glucocorticoids, vascular endothelial growth factor A (VEGF-A), and β-FGF, as well as estrogen and cardiovascular burden. The biological functions of OPN can be modified by matrix metalloproteases (MMP 2, 3, 9) and thrombin [2]. OPN contains hematopoietic cells in the bone marrow compartment close to the endosteal zone while keeping these cells in a quiescent state (Figure 1) [17]. The interesting observation is that OPN in the bone marrow and in the vascular endothelium is an important player in the E2-stimulated EPC homing [18].

Recent studies emphasize the strong correlation between bone matrix that is secreted during bone tissue regeneration and angiogenesis. One of the most important components of bone matrix is osteopontin that is linked to the bone regeneration and angiogenesis. Osteopontin serves an adhesive substrate for both vascular smooth muscle (SMCs) and endothelial cells as well a potent chemo tactic factor for SMCs [19]. OPN mRNA is present in a plaque and mainly expressed in macrophages but also in smooth muscle cell and endothelial cells [20]. OPN is correlated with bone turnover and remodeling and may play a role in plaque calcification. The literature reported the expression of bone morphogenic protein 2a, a potent factor for osteoblast differentiation, in human atherosclerosis suggesting that the plaque calcification is an active process [21]. Osteoblasts regulate bone formation and its precursors come from the mesenchyma of bone marrow while osteoclasts come from hematopoietic precursors. Both osteoclasts and osteoblasts produce OPN and their activity is crucial for bone remodeling besides osteoid mineralization (Figure 1).

3 Hematopoetic Stem Cells

OPN protein is abundantly expressed in a very restricted zone at the endosteal interface of bone and hematopoietic tissue. Osteoblasts within this zone have been proposed as key components of the hematopoietic stem cells' (HSC) niche and implicated in regulating HSC numbers, both in vitro and in vivo [22-24]. This may be mediated in part by direct cell-cell contact, whereby angiopoietin-1 or membrane-bound Jagged1 on osteoblasts elicits signaling of Tie2 or notch1 expressed on HSCs, respectively, resulting in an increased number of HSCs [17]. Within the hematopoietic niche the main source of OPN is likely to be osteoblasts lining bone surfaces within the bone marrow cavity. It seems that osteoblasts exert both inhibitory and stimulatory effects on HSC proliferation and differentiation via different and or overlapping mechanisms. OPN is a key component of the HSC niche (Figure 2). It exhibits a highly restricted pattern of expression at the endosteal surface and contributes to HSC trans-marrow migration toward the endosteal region after transplantation, and has an important physiologic role in the regulation of HSC location and proliferation (Figure 3) [17].

Proliferation of bone marrow cells co-cultured with MED1 knockout mice embryonic fibroblasts was suppressed compared to controls. The vitamin D expression of OPN was specifically attenuated in these cells, but addition of OPN restored the growth of the co-

cultured bone marrow cells [25]. An in-vitro model has demonstrated that OPN secreted by glioma cells accelerated endothelial progenitor cells angiogenesis in vitro, including tube formation. OPN also induced activation of AKT endothelial nitric oxide synthase and increased nitric oxide production and angiogenesis in endothelial progenitor cells. Taken together, these data showed that OPN directly stimulates angiogenesis through the PI3-K/AKT/eNOS/NO signaling pathway [26].

4 Mesenchymal Stem Cells (MSCs)

MSCs represent a small percentage of bone marrow cells, and they can be partially distinguished from hematopoeitic cells by their ability to adhere to tissue culture dishes [27]. In culture, they can differentiate into bone, fat, cartilage, or muscle cells using specific media [28-31]. MSCs also have vascular differentiation potential. In culture, they differentiate into smooth muscle cells (SMCs) [32, 33]. In vivo, bone marrow–derived cells that were seeded on a synthetic vascular graft produced smooth muscle and endothelial layers [34]. Vascular SMCs were described as "multifunctional mesenchymal cells" 36 years ago [35]. Moreover, in atherosclerotic lesions both vascular SMCs and macrophages express OPN and matrix Gla protein [36]. It has been hypothesized that in the healthy vessel wall calcification is inhibited by inhibitor proteins but if inhibition is lost or when the equilibrium between inhibition and initiation of calcification is disturbed, the vessel wall will calcify [37, 38]. Thus vascular SMCs express many of the calcification-regulating proteins commonly found in bone. The precise role of these proteins in the calcification process is not clear yet. However, many of these proteins have calcium and apatite binding properties and accumulate in areas of vascular calcification. Besides vascular SMCs, pericytes have been linked to vascular calcification [39]. Pericytes are located in the microvasculature, where they share a basement membrane with endothelial cells and contribute to the "tightness" of capillary permeability. Pericytes are also involved in angiogenesis [40]. Evidence is accumulating that pericytes can function as progenitor cells and that pericytes are capable of differentiating into osteoblasts, chondrocytes, adipocytes, and SMCs [41, 42]. Pericyte-like cells have been found in the inner intima, outer media and vasa vasorum of arteries and may play a role in the calcification process observed during atherosclerosis (Figure 4).

5 The Effects of OPN on Blood Vessels

OPN is expressed in proliferating and migratory vascular cells associated with neointima formation and in inflammatory cells. In human atherosclerotic lesions, OPN is expressed in smooth muscle cells in the lesion, in angiogenic endothelial cells, and in macrophages and is associated with mineralized deposits in humans [19].

Vascular calcification is now recognized as a marker of atherosclerotic plaque burden as well as a major contributor to loss of arterial compliance and increased pulse pressure seen with age, diabetes mellitus, and renal insufficiency. OPN together with other bio-mineralization inhibitors may control whether calcification occurs under these pathological conditions (Figure 5). Mechanistically increasing evidence suggests that

phosphorylated OPN directly associates with apatite deposits and blocks crystal growth in addition to inducing RGD-mediated mineralization of cardiovascular tissues [42-44].

6 OPN and Endothelial Cells

OPN protects endothelial cells from apoptosis induced by growth factor withdrawal. This interaction is mediated by the $\alpha_v\beta_3$ integrin and is Nuclear Factor kappa B (NF-κ-B) dependent. OPN is affecting endothelial cells through activation of genes one of them is the osteoprotegerin, a member of the tumor necrosis factor receptor super-family. Levels of osteoprotegerin mRNA and protein were low in endothelial cells that were not attached with integrins, however, their level was increased 5-7 fold following $\alpha_v\beta_3$ ligation by OPN. Osteoprotegerin induction by OPN was time dependent. NF-κ-B inactivation in endothelial cells inhibited osteoprotegerin induction by OPN. These data suggest that $\alpha_v\beta_3$ mediated endothelial survival depends on osteoprtegerin induction by NF-κ-B and indicate a new function for osteoproetgerin and for OPN in endothelial cells [45]. After balloon catheter denudation, OPN mRNA levels correlated temporally and spatially with active endothelial proliferation and migration, with the highest levels observed at the wound edge between 8 hours and 2 weeks after injury, declining to uninjured levels at 6 weeks, when regeneration was complete (Figure 6). OPN protein levels, as determined by immune-cytochemistry, paralleled the time course of mRNA expression. Likewise, β_3-integrin mRNA and protein levels were substantially elevated in regenerating endothelial cells but were not detectable in uninjured or healed endothelium. These data suggest important roles for OPN and β_3 integrin in regenerating endothelium [6].

Bone development requires the recruitment of osteoclast precursors from the surrounding mesenchymes, thereby allowing the key events of bone growth such as marrow cavity formation, capillary invasion, and matrix remodeling. Matrix metalloproteinase 9 (MMP-9) is specifically required for the invasion of osteoclasts and endothelial cells into the discontinuously mineralized hypertrophic cartilage that fills the core of the diaphysis. MMP-9 stimulates the solubilization of un-mineralized cartilage by MMP-13, a collagenase highly expressed in hypertrophic cartilage before osteoclast invasion. Hypertrophic cartilage also expresses vascular endothelial growth factor (VEGF), which binds to extracellular matrix and is made bio-available by MMP-9. MMP-9 and VEGF have both specific and critical roles for early bone development [46].

7 The Paracrine Effects of Osteopontin

Osteopontin (OPN) is a secreted adhesive molecule, thought to aid in the recruitment of monocyte-macrophages and to regulate cytokine production in macrophages, dendritic cells, and T cells. OPN has been classified as T-helper 1 cytokine and thus is believed to exacerbate inflammation in several chronic inflammatory diseases, including atherosclerosis. OPN is a potent inhibitor of mineralization; it prevents ectopic calcium deposits and is a potent inducible inhibitor of vascular calcification. Its effect is believed to function through its adhesive domains, especially the Arginine-Glycine-Aspartate (RGD) sequence that interacts with several integrin heterodimers [2]. Several studies have shown that OPN is cleaved by at least 2 classes of proteases: thrombin and matrix

metalloproteases (MMPs) [2]. OPN acts both as a matricellular protein, thereby facilitating adhesion and migration, as well as a soluble cytokine [3]. Several in vitro studies indicated that OPN induces adhesion, migration, and survival of several cell types, including smooth muscle cells, endothelial cells, and inflammatory cells [4, 6]. In vivo studies of OPN in disease and injury have suggested an important function for this molecule in inflammation and tissue remodeling [5]. In particular, OPN is de novo expressed in cells participating in renal and cardiovascular system during remodeling and repair processes [19, 47, 48] and is highly expressed in inflammatory cells associated with cancer, arterial re-stenosis, myocardial infarction, stroke, and wound healing (20, 49, 50). Wound healing studies also indicated that OPN is expressed during the acute inflammatory phase at very high levels in infiltrating leukocytes. Other studies in bone wound healing suggest that OPN is a positive regulator of phagocytic activity [51]. OPN also affects lymphocytic phenotype, and it was cloned from activated CD4+ cells, where it is highly expressed [52]. OPN induces T cell chemotaxis, supports T cell adhesion and co-stimulates T cell proliferation [53]. It also induces CG40 ligand and IFN gamma expression on human T cells, resulting in CV40L-and INF dependent IL-12 production concomitantly with CD3 stimulation. These findings suggest a functional role for OPN in early Th1 responses, namely regulation of T cell-dependent IL-12 production [54].

8 Summary

Bone regeneration is a result of complex processes of mesenchymal stem cells' invasion, chondrogenesis, osteogenesis and angiogenesis. The coordinated actions of these principles processes result in the reconstruction and restoration of a structural unit. Recent discoveries of circulating multi-potent stem cells with mixed characteristics of endothelial cell and osteogenic capacity have raised interest in new and potentially breakthrough therapies for fracture and pathologic bone loss.

Long bones epiphyses are the home of hematopoietic stem cells and serve as the niche for these stem cells. Recent studies emphasize the close relationship between hormones that are secreted from bones and angiogenesis. Hormones and peptides affect the angiogenic system through activation of different stem cells and transcriptional factors. Calcification of blood vessels, used now as a marker of atherosclerosis, altogether with bone regeneration processes are all connected.

A better understanding of the mechanism that link between bones and blood vessels may open a new frontier to fight atherosclerosis and enhance blood vessel repair.

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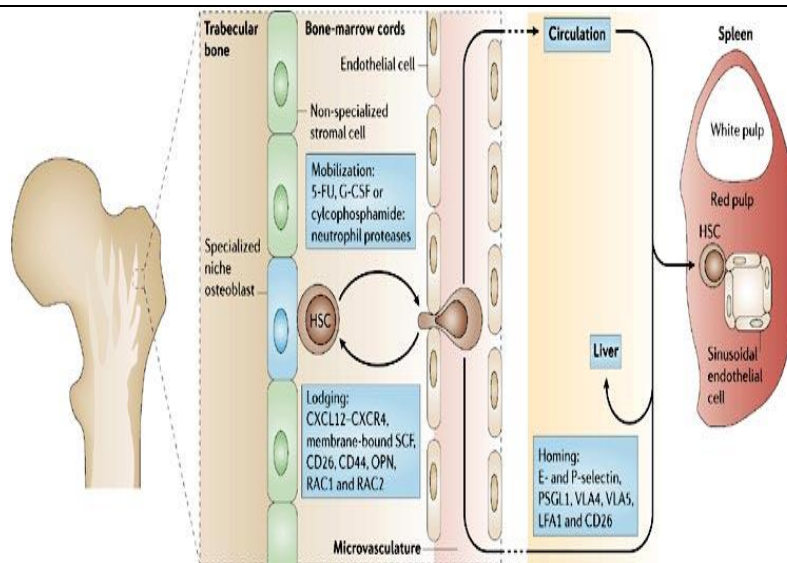
Appendix

Figure 1: Stem Cells Niche within the Epiphysis

Stem cell niche within the epiphysis and its relationships with other organs: endothelial cells, receptors and peptides.
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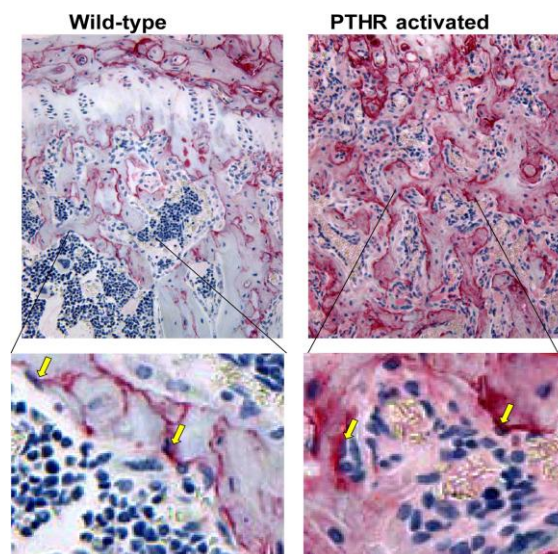

Figure 2: Osteopontin is a hematopoietic stem cell niche component

OPN is increased in bone marrow with the activation of osteoblasts: Immunohistochemistry of tibia sections from wild-type (left) or transgenic (right) mice with a constitutively activated PTH/parathyroid related peptide receptor. Sections were stained with antibody to osteopontin (red). Yellow arrows - OPN-rich spindle shaped cells lining the trabecular bone, consistent with an osteoblast morphology. Reprinted with permission from the Rockefeller University Press; Journal of Experimental Medicine 201(11): 1781-1791, 2005.
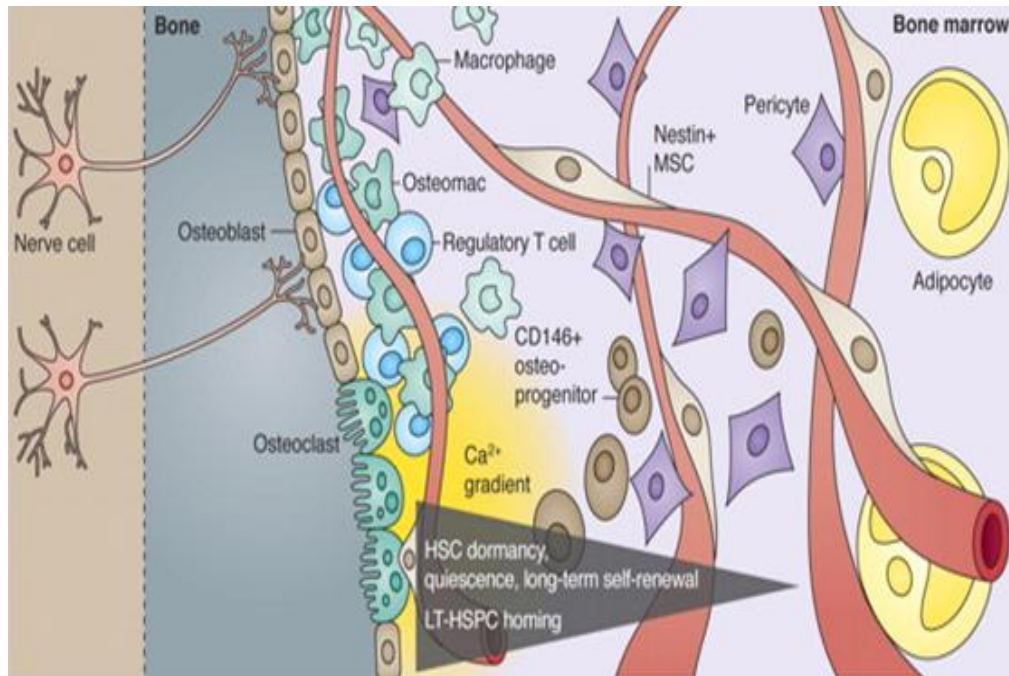
Figure 3: The HSC microenvironment

The micro-environment of the stem cell niche – T cells, receptors, and bone cells homeostasis. Reprinted with permission from the Journal of Cell Science (jcs.biologists.org.) vol. 124: 3529-3535, 2011
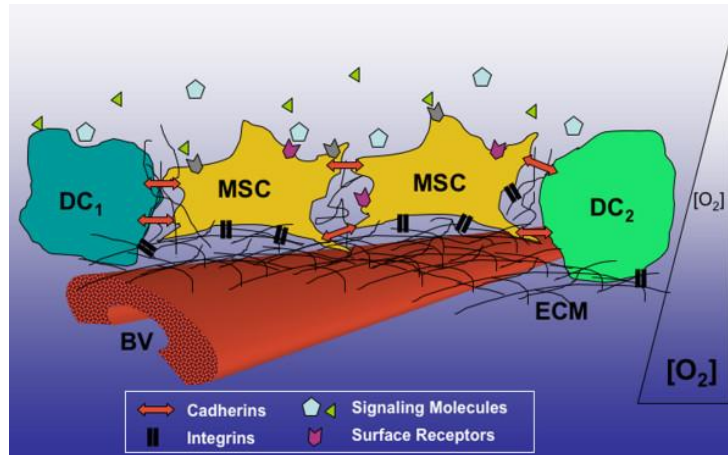
Figure 4: Mesenchymal stem cell nyche

Mesenchymal stem cell niche. Mesenchymal stem cells (MSCs) are shown in their putative perivascular niche (BV, blood vessel), interacting with various other differentiated cells (DC$_1$, DC$_2$, etc.) by means of cell-adhesion molecules, such as cadherins, extracellular matrix (ECM) deposited by the niche cells mediated by integrin receptors, and signaling molecules, which may include autocrine, paracrine, and endocrine factors. O$_2$ tension, with hypoxia associated with MSCs in the bone marrow niche. Reprinted with permission from Arthritis Research and Therapy 9(1): 204, 2007
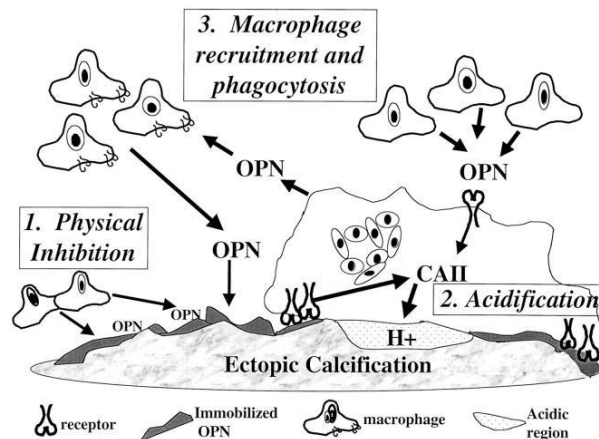

Figure 5: Osteopontin Inhibits Mineral Deposition

Mechanism of osteopontin action in regulating ectopic calcification: It is proposed that OPN made by stromal or inflammatory cells at sites of ectopic mineralization binds to mineral and initially inhibits crystal growth. Reprinted by permission from the American Society for Investigative Pathology, published in the American Journal of Pathology vol. 161 (6): 2035-2046, 2002.
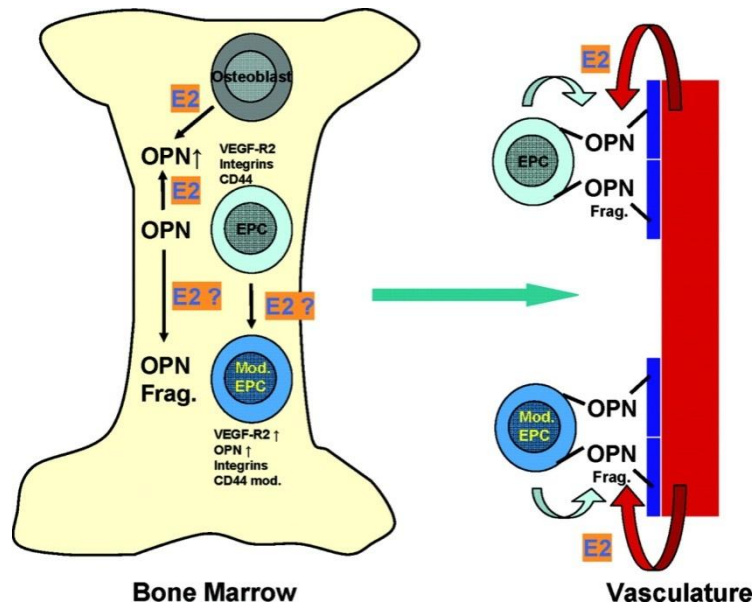
Figure 6: Mechanisms of Estrogen-induced OPN-dependent EPC homing to sites of vascular injury

OPN and OPN-fragments and vascular regeneration - serve as linkers between EPCs and vascular endothelium through their capacity to bind to integrins and CD44. Osteoblasts, EPCs, as well as vascular cells serve as the source for OPN. Estrogen (E2) might stimulate this OPN-dependent homing of EPCs through modification of EPC-differentiation (mod EPC) in the bone marrow compartment; for instance by increasing the expression of VEGF-R2 or by stimulating the production of OPN and, subsequently, release of OPN by residing osteoblasts. Estrogen also might stimulate the production of OPN-fragments through stimulation of MMP activity, increasing the binding capacity of modified EPC to the injured vessel wall. Reprinted with permission from Arteriosclerosis, Thrombosis, and Vascular Biology 28: 2099-2100, 2008