Biochimica et Biophysica Acta 1855 (2015) 202–222

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbacan

Review

Interactions between osteopontin and vascular endothelial growth factor: Implications for cancer

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ARTICLE INFO

Article history:
Received 29 November 2014
Received in revised form 10 February 2015
Accepted 22 February 2015
Available online 28 February 2015

Keywords:
Angiogenesis
Hypoxia
Apoptosis
Cancer
Vascular disease
Immune system

ABSTRACT

For this comprehensive review, 257 publications with the keywords "osteopontin" or "OPN" and "vascular endothelial growth factor" or "VEGF" in PubMed were screened (time frame from year 1996 to year 2014). 37 articles were excluded because they were not focused on the interactions between these molecules, and papers relevant for transformation-related phenomena were selected. Osteopontin (OPN) and vascular endothelial growth factor (VEGF) are characterized by a convergence in function for regulating cell motility and angiogenesis, the response to hypoxia, and apoptosis. Often, they are co-expressed or one molecule induces the other, however, in some settings OPN-associated pathways and VEGF-associated pathways are distinct. Their relationships affect the pathogenesis in cancer, where they contribute to progression and angiogenesis and serve as markers for poor prognosis. The inhibition of OPN may reduce VEGF levels and suppress tumor progression. In vascular pathologies, these two cytokines mediate remodeling, but may also perpetuate inflammation and narrowing of the arteries. OPN and VEGF are elevated and contribute to vascularization in inflammatory diseases.

Contents

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http://dx.doi.org/10.1016/j.bbcan.2015.02.003
0304-419X/

1. Introduction

Vascular endothelial growth factor (VEGF) and osteopontin (OPN) are secreted molecules with cytokine properties. Past studies have found that under specific conditions these proteins may be co-expressed [1–10], OPN can induce VEGF [11–21], or VEGF can induce OPN [22–32], and both molecules can function in synergy. In other cases, OPN and VEGF act mutually independently [33–51]. Rarely, one suppresses the expression or function of the other [52–55]. To elucidate the modes of communication between VEGF and OPN, and the conditions under which such interactions occur, we review the published literature on both molecules (Table 1). We identify physiologic and cancer-related pathophysiologic states, for which interactions between OPN and VEGF have been studied. To cover the existing knowledge basis, connections between OPN and VEGF in the skeletal and renal system are reviewed elsewhere [56].

2. Physiologic functions regulated by OPN and VEGF

2.1. Cell motility and angiogenesis

Angiogenesis, the formation of new blood vessels, is important both in normal physiology, such as wound healing, and in pathological states, such as tumor growth. It involves cell migration as an essential contributing component. VEGF and OPN can mediate cell migration through chemotaxis or haptotaxis, and both molecules may support angiogenesis.

2.1.1. Co-expression of OPN and VEGF

Functional angiogenesis in the skeletal muscle requires OPN and VEGF. The latter alone (pathological angiogenesis via induction by HIF-1α) induces the formation of a disorganized vasculature. Hypoxia up-regulates OPN expression, and the hypoxia-dependent transcription factor HIF-1α induces the expression of VEGF. Together, they stimulate angiogenesis in osteogenic differentiation [57], myocardial ischemia [14], pulmonary hypertension [58,59], and diabetic retinopathy [60]. Both OPN and VEGF have significantly elevated expression in the synovial fluid of patients with juvenile idiopathic arthritis. The secretion of OPN correlates positively with VEGF concentration as well as vascularization of the inflamed synovial tissue [61]. VEGF and OPN co-expression is associated with angiogenesis and unfavorable outcome in various cancers. Specifically, high levels of both proteins correlate with angiogenesis and poor prognosis in lung adenocarcinoma [62], gastric carcinoma [63], myeloid leukemia [64], breast cancer [11], and prostate cancer [20]. The down-regulation of both VEGF and OPN by the bicyclic diterpenoid lactone andrographolide may inhibit tumor-endothelial interaction, thus suppressing cancer cell growth and metastasis [65]. Cells of the immune system contribute to the formation of new blood vessels. Through the exposure to dendritic cells, stem/progenitor cells become enriched with endothelial progenitor cells, which have a potential for both angiogenesis and stemness, and secrete OPN and VEGF along with interleukin-8 and interleukin-10 [66]. Myocytes over-expressing peroxisome proliferator-activated receptor γ co-activator 1α (PPARGC1A, PGC-1α) activate VEGF-A and secrete OPN, which leads to MCP-1 production by macrophages, causing the activation of endothelial cells and pericyte migration, thus inducing functional angiogenesis [67].

2.1.2. Induction of VEGF by OPN

The OPN/VEGF axis is important for endothelial cell motility, proliferation, and tube formation. It is dependent on the interaction of OPN with integrin $\alpha_v\beta_3$, which confers cyto-protection to endothelial cells through the activation of the PI3K/AKT pathway with subsequent up-regulation of BCL-X_L and activation of NF-κB. The phosphorylation of AKT and ERK also enhances VEGF expression. In turn, the OPN-induced VEGF activates the PI3K/AKT and ERK1/2 pathways via a positive feedback signal [16] (Fig. 1A). In ocular fibroblasts, OPN enhances TGF-β1 mediated signaling via SMAD3 and the kinase P38 and increases VEGF expression, leading to corneal neovascularization in response to an injury [68]. OPN is induced and causes an increase in VEGF levels with consecutive angiogenesis during myocardial ischemia [14] and in the ischemic retina [69]. Laser irradiation up-regulates OPN in choroidal tissue (the vascular layer of the eye), which prompts enhanced VEGF expression and macrophage infiltration, thus aiding in choroidal neovascularization [70]. Tumor-derived OPN can induce the expression of VEGF. In malignant pleural effusions associated with advanced stage lung adenocarcinoma, VEGF secretion by mesothelial and endothelial cells is increased upon OPN stimulation [15]. OPN also triggers VEGF-dependent tumor progression and angiogenesis through autocrine and paracrine mechanisms in breast cancer [11]. Tumor-derived OPN induces VEGF expression via BRK- and NIK-mediated activation of ATF-4, leading to tumor cell migration in an autocrine manner [11] (see Fig. 3). In neuroblastoma cells, however, tumor-derived OPN does not increase VEGF production and does not affect the gene expression of other angiogenic factors, although it induces the migration of vascular endothelial cells resulting in angiogenesis [51].

OPN undergoes alternative splicing, a typical characteristic of metastasis genes. Three distinct forms of the protein are generated. In lung cancer, overexpression of the full-length form OPNa is correlated to higher VEGF secretion and increased tubule length over the splice variants OPNb and OPNc [18]. On the other hand, among all OPN splice forms, OPNc induces higher levels of VEGF in prostate cancer cells, followed by OPNb while OPNa does not have this effect [20] (Fig. 2). Osteopontin splicing has also been described in cancers of the breast, esophagus, stomach, brain, head and neck, liver, pancreas, ovaries and endometrium, and in soft tissue sarcomas. However, interactions of the variant forms with VEGF have not been elucidated in those malignancies.

2.1.3. Induction of OPN by VEGF

VEGF up-regulates the levels of proteins that are important for vascular remodeling. Specifically, it increases the expression of OPN and other extracellular matrix proteins by endothelial cells [54], and the expression of OPN along with the integrin subunits α_v and β_3, by umbilical artery cells [32] and dermal microvascular endothelial cells

Table 1
Literature on OPN and VEGF.

Reference	State/disease	Tissue	Cells	Induction	Condition/treatment
[36]	Angiogenesis	Blood vessels	Endothelial cells	Distinct pathways	Effect of sonic hedgehog
[70]	Angiogenesis	Eye		OPN induces VEGF	Laser irradiation
[72]	Angiogenesis	Immune system	Macrophage-like cell	Distinct expression	Poly(-methacrylic acid-co-methyl methacrylate)
[66]	Angiogenesis	Blood vessels	Co-culture: stem/progenitor cells, endothelial progenitor cells	Higher levels	Treatment for vascular diseases
[158]	Angiogenesis	Heart		Higher levels	Valvular calcification, rheumatic heart disease
[33]	Angiogenesis	Blood vessels	Endothelial cells	Distinct pathways	
[74]	Angiogenesis	Blood vessels	Endothelial cells	OPN in angiogenesis	
[75]	Angiogenesis	Blood vessels	Endothelial cells	OPN in angiogenesis	
[198]	Angiogenesis, bone regeneration	Bone	Murine preosteoblastic cells	Higher levels	Incorporation of bioactive inorganic particles
[39]	Angiogenesis, bone-mineral metabolic disorders	Bone/kidney		Distinct pathways	Matrix extracellular phosphoglycoprotein overexpression
[28]	Angiogenesis, cancer	Brain		VEGF induces OPN	Glioblastoma, malignant
[29]	Angiogenesis, cancer	Brain		VEGF induces OPN	Glioblastoma, malignant
[37]	Angiogenesis, cancer	Blood vessels	Endothelial progenitor cells, human glioma cells	Distinct pathways	Glioma
[11]	Angiogenesis, cancer	Breast	Breast adenocarcinoma cells	OPN induces VEGF	
[46]	Angiogenesis, cell motility	Kidney	Kidney endothelial and epithelial cells	Distinct expression	Bim deficiency
[61]	Angiogenesis, inflammation	Joint		Higher levels	Arthritis, juvenile idiopathic
[104]	Angiogenesis, inflammation	Lung	LLC, B16F10 melanoma, RAW 264.7 macrophage, pNGL LLC26 and HPMEC-ST1 endothelial cell	Distinct pathways	Malignant pleural effusion
[88]	Apoptosis	Mouth	Dental pulp stem cells	Higher levels	Treatment with NAC
[170]	Bone regeneration	Bone	Mesenchymal stem cells	Higher levels	Allogeneic mesenchymal stem cell transplantation
[197]	Bone regeneration	Bone	Osteoblasts	Higher levels	Low intensity pulsed ultrasound
[164]	Bone regeneration	Bone		Higher levels	Marrow ablation, femoral
[209]	Bone regeneration	Mouth	Periodontal ligament and Endothelial cells	Upregulated	Periodontal tissue, effect of BDNF
[169]	Bone regeneration	Bone	Murine preosteoblastic cells	Higher levels	Thermal stress conditioning and growth factors
[1]	Bone regeneration	Bone	Circulating marrow-derived osteoblast progenitor cells	Co-expression	
[165]	Bone remodeling	Bone		Higher levels	Stress fracture healing
[163]	Bone repair	Bone		Higher levels	Fractures, long bone
[25]	Bone repair	Bone	Bone marrow stromal cells	VEGF induces OPN	
[193]	Bone repair	Bone		Higher levels	Distraction osteogenesis
[194]	Bone repair	Bone	Primary human osteoblast cells	Higher levels	Distraction osteogenesis
[195]	Bone repair	Bone		Higher levels	Distraction osteogenesis, mandibular
[203]	Bone repair, angiogenesis	Bone		Higher levels	Fractures, treatment with formononetin
[139]	Brain tumors, ossification	Brain		Expressed	

[95]	Cancer	Blood		Higher levels	Acute leukemia
[5]	Cancer	Colorectum	colorectal adenocarcinoma cells	Correlated expression	Adenocarcinoma
[62]	Cancer	Lung		Higher levels	Adenocarcinoma
[15]	Cancer	Lung	Lung cancer cells, umbilical vein endothelial cells, mesothelial cells	OPN induces VEGF	Adenocarcinoma, advanced stage
[7]	Cancer	Lung		Co-expression	Adenocarcinoma, pulmonary tumor thrombotic microangiopathy
[152]	Cancer		Human umbilical vein endothelial cells	Higher levels	Antibody against Endoglin
[150]	Cancer	Breast, liver	Human hepatocellular carcinoma cell lines, breast carcinoma cell line and Chinese hamster ovary	Inhibition	Bispecific antibody against OPN and VEGF
[109]	Cancer	Breast		VEGF suppression	Blockade of OPN-receptor binding
[50]	Cancer	Stomach	Gastric cancer cell line	Distinct pathways	Carcinoma, effect of OPN siRNA
[110]	Cancer	Stomach	Gastric cancer cells	Reduced levels	Carcinoma, effect of OPN siRNA
[63]	Cancer	Stomach		Upregulated	Carcinoma, primary and PTTM lesions
[130]	Cancer	Stomach		Upregulated	Carcinoma, primary and PTTM lesions
[131]	Cancer	Stomach		Upregulated	Carcinoma, primary and PTTM lesions
[49]	Cancer	Placenta		Distinct expression	Chorioangioma
[127]	Cancer	Bladder		Higher levels	Clear cell renal cell carcinoma
[47]	Cancer	Liver		Distinct expression	Colorectal cancer, metastasis
[129]	Cancer	Stomach	Gastric cancer cell line	Reduced levels	Downregulation of COX-2
[113]	Cancer	Lung	Lung cancer cells	Higher levels	Downregulation of Tip30
[19]	Cancer	Pancreas		OPN induces VEGF	Ductal adenocarcinoma/effect of nicotine
[65]	Cancer	Breast	Breast adenocarcinoma cells	OPN and VEGF suppression	Effect of andrographolide
[116]	Cancer	Breast	Breast adenocarcinoma cells	OPN and VEGF suppression	Effect of berbamine
[112]	Cancer	Breast	Breast adenocarcinoma cells	OPN and VEGF suppression	Effect of curcumin
[114]	Cancer	Lung	Non-small cell lung cancer cells	Reduced levels	Effect of ethanol extract of *Ocimum sanctum*
[99]	Cancer	Stomach		Higher levels	Gastric carcinoma
[100]	Cancer	Stomach		Higher levels	Gastric carcinoma
[136]	Cancer	Liver	HCC cell lines	Reduced levels	Gene therapy with IFN-α and SG600-IL-24
[138]	Cancer	Brain	Glioma, glioblastoma, leukemic T-cell lymphoblast	Positively correlated	Glioblastoma, malignant
[140]	Cancer	Head		Distinct pathways	Head and neck cancer
[93]	Cancer	Liver		Upregulated	Hepatocellular carcinoma
[105]	Cancer	Liver		Upregulated	Hepatocellular carcinoma
[107]	Cancer	Liver		Upregulated	Hepatocellular carcinoma
[134]	Cancer	Liver		Distinct expression	Hepatocellular carcinoma, chronic hepatitis
[98]	Cancer	Lung	Non-small cell lung cancer cells, fibroblasts	Secreted	IGF1R-stimulation
[108]	Cancer	Lung		Reduced levels	Lewis lung carcinoma, effect of topiramate
[102]	Cancer	Pancreas		Upregulated	Liver metastasis of pancreatic cancer
[124]	Cancer	Prostate		Upregulated	Lymph node metastases
[9]	Cancer	Lung		Correlated expression	Malignant exudative pleural effusion
[144]	Cancer	Skin	Melanoma cell line	Reduced levels	Melanoma, treatment with lang-du extract
[115]	Cancer	Lung		Upregulated	Mesothelioma
[96]	Cancer	Blood		Higher levels	Multiple myeloma
[106]	Cancer	Blood		Higher levels	Multiple myeloma
[97]	Cancer	Blood		Higher levels	Multiple myeloma, stage III
[76]	Cancer	Blood		Higher levels	Myeloid leukemia
[51]	Cancer	Nervous system	Neuroblastoma cells	Distinct pathways	Neuroblastoma
[8]	Cancer	Lung		Expressed along with Tenascin-C	Non-small cell
[90]	Cancer	Lung		Upregulated	Non-small cell
[92]	Cancer	Lung		Prediction of outcome by OPN, not VEGF	Non-small cell, advanced
[137]	Cancer	Pancreas		Upregulated	Osteoclast-like giant cell tumors
[145]	Cancer	Bone	Osteosarcoma cells	Distinct pathways	Osteosarcoma
[3]	Cancer	Bone		Higher correlated expression	Osteosarcoma
[4]	Cancer	Breast		Co-expression	Osteosarcoma, primary
[146]	Cancer	Bone	Osteosarcoma, primary	Lower levels	Osteosarcoma/Zolendronate treatment
[135]	Cancer	Liver	HCC cell lines	Reduced levels	PARP-1 inhibition
[31]	Cancer	Stomach		VEGF induces OPN	Role of NR4A2
[125]	Cancer	Prostate	Prostate cancer cells	Upregulated	Role of Runx2

(continued on next page)

Table 1 (*continued*)

Reference	State/disease	Tissue	Cells	Induction	Condition/treatment
[126]	Cancer	Prostate	Prostate cancer cell line	Suppressed	Role of SSeCKS
[142]	Cancer	Head		Higher levels	Sinonasal inverted papilloma
[117]	Cancer	Breast	Breast adenocarcinoma cells	Reduced levels	siRNA (gycerol propoxylate triacrylate and spermine, GPT-SPE, as gene carrier) against OPN
[151]	Cancer	Various	Epidermoid carcinoma, colorectal adenocarcinoma, prostate cancer, glioma	Higher levels	Treatment with sunitinib
[94]	Cancer	Bladder		Higher levels	Urothelial cell carcinoma
[40]	Cancer	Breast		Distinct expression	
[78]	Cancer	Prostate		Distinct roles	
[123]	Cancer	Cervix		Higher levels	
[132]	Cancer	Colorectum		Higher levels	
[133]	Cancer	Colorectum		Inverse correlation	
[57]	Cancer	Prostate	Prostate tumor Endothelial cells	OPN and VEGFR-2 upregulated	
[118]	Cancer	Ovaries		OPN is a better marker than VEGF	
[18]	Cancer	Lung		OPNa induces VEGF	
[20]	Cancer	Prostate	Prostate cancer cells	OPNc and OPNb induce VEGF	
[121]	Cancer	Ovaries		Over-expressed	
[143]	Cancer	Skin	Melanoma cell line	Over-expressed	
[119]	Cancer	Ovaries and pancreas		Upregulated	
[120]	Cancer	Ovaries		VEGF is a better marker than OPN	
[111]	Cancer	Colon	Colon cancer cell line	VEGF suppression via OPN downregulation	
[103]	Cancer	Blood		Higher levels	Chronic lymphocytic leukemia
[128]	Cancer	Bladder		Higher levels	Metastatic renal cell carcinoma
[147]	Cancer	Blood		Higher levels	Multiple myeloma
[148]	Cancer	Blood		Higher levels	Multiple myeloma
[149]	Cancer	Blood		Lower levels	Multiple myeloma
[173]	Cell differentiation	Bone	Human articular chondrocytes	Higher levels in 2D	2D vs. 3D culture
[210]	Cell differentiation	Mouth, bone	Dental pulp stem cells, primary osteoblasts	Higher levels	Biocoral scaffolds
[174]	Cell differentiation	Bone	Bone marrow stromal cells	Higher levels	BMP-2 treatment, 2D vs. 3D culture
[38]	Cell differentiation	Epithelial cells	Keratinocytes	Distinct pathways	Effect of calcium ion cyclotron resonance
[87]	Cell differentiation	Bone	Adipose-derived stem cells	Differential responses	Hypoxia
[201]	Cell differentiation	Bone	Bone marrow stromal cells	Higher levels	Osteogenesis, tooth extraction
[175]	Cell differentiation	Bone	Bone marrow stromal cells	Differential responses	
[32]	Cell motility, angiogenesis	Umbilical arteries	Umbilical vein endothelia cells	VEGF induces OPN	Healing
[16]	Cell survival, motility, angiogenesis	Breast	Umbilical vein endothelia cells	OPN induces VEGF and vice versa	
[168]	Delayed ossification, differentiation	Bone	Chondrogenic cells	Lower levels	Deletion of TGFβ type II receptor gene
[204]	Fracture healing	Bone		Higher levels	Ultralente insulin
[53]	Hemorrhage	Brain		VEGF suppressed by OPN	Sub-arachnoid hemorrhage
[208]	Homeostasis	Mouth	Malassez's epithelial rest cells	Lower levels	Treatment with NGF vs. EGF
[14]	Hypoxia	Heart	Primary hepatocytes	OPN induces VEGF	Ischemia
[79]	Hypoxia	Mouth	Cementoblasts	Higher levels	
[69]	Hypoxia, Angiogenesis	Eye, Retina	Retinal endothelial cells	Higher levels	Ischemia-induced neovascularization
[12]	Hypoxia, apoptosis, cancer	Breast	Breast adenocarcinoma cells	OPN induces VEGF	
[84]	Hypoxia, apoptosis, cancer	Breast		OPN induces VEGF	
[85]	Hypoxia, apoptosis, cancer	Breast	Breast adenocarcinoma cells	VEGF suppression via OPN downregulation	
[141]	Hypoxia, cancer	Head		Higher levels correlated with HIF-1α	Head and neck cancer, radio(chemo)therapy
[91]	Hypoxia, cancer	Lung		Upregulated	Non-small cell, stage M0
[86]	Hypoxia, cancer	Skin	Fibrosarcoma, squamous cell carcinoma and melanoma cell lines	No expression	
[64]	Hypoxia, cancer	Blood	Myeloid leukemia cells, primary	Higher levels	Acute myeloid leukemia
[77]	Hypoxia, cancer	Brain	Glioblastoma cells	Higher levels	Glioblastoma
[6]	Hypoxia, cancer	Head		Correlated expression	Head and neck cancer
[101]	Hypoxia, cancer	Head		Higher levels	Head and neck cancer
[80]	Hypoxia, inflammation	Joint	Mononuclear cells	Higher levels	Arthritis, juvenile idiopathic

[58]	Hypoxia, inflammation	Lung	Leukocytes	Higher levels	Pulmonary hypertension
[44]	Hypoxia, kidney disease	Kidney		Distinct expression	Chronic disease
[83]	Hypoxia, osteogenesis	Bone	Bone marrow stromal cells	Higher levels	Effect of dimethyloxallyl glycine
[81]	Hypoxia, osteogenesis	Bone	Mesenchymal stromal cells	Higher levels	Restoration of bone function
[172]	Hypoxia, tissue engineering	Bone	Bone marrow-derived mesenchymal stromal cells	Higher levels	Hypoxia + bone-derived scaffolds
[17]	Immune system	Lung	Lung fibroblasts	OPN induces VEGF	Airway remodeling, ovalbumin-induced/asthma
[160]	Immune system	Blood	Human monocytic leukemia cells	Higher levels	*Mycobacterium tuberculosis* infection
[162]	Immune system	Immune system		Distinct expression	Presence of autoantibodies
[48]	Immune system	Lung		Distinct expression	TGF-α-induced pulmonary fibrosis
[161]	Inflammation	Uterus		Distinct expression	
[159]	Inflammation, airway remodeling	Lung		Higher levels	Aerobic training
[71]	Inflammation, angiogenesis	Lung	Lung Endothelial cells	Distinct expression	PEDF deficiency
[68]	Injury	Eye, cornea	Ocular fibroblasts	VEGF suppression via OPN downregulation	Neovascularization in corneal stroma
[215]	Kidney disease	Kidney		Higher levels	Chronic proteinuria
[218]	Kidney disease	Kidney		Distinct expression	Drug-induced
[45]	Kidney disease	Kidney	podocytes	Distinct expression	Focal segmental glomerulosclerosis
[214]	Kidney disease	Kidney		Higher levels	Nephrogenic systemic fibrosis, gadodiamide-induced
[52]	Kidney disease	Blood vessels		VEGF suppresses OPN	Post-cyclosporin nephropathy and hypertension
[55]	Kidney disease	Kidney		VEGF suppresses OPN	Renal fibrosis/treatment with VEGF
[217]	Kidney disease	Kidney		Higher levels	Renal pathologies, drug-induced/chronic nephrotoxicity
[219]	Kidney injury	Kidney		Distinct expression	Aristolochic acid-induced renal injury
[213]	Kidney injury, acute	Kidney		Upregulated	
[216]	Kidney transplantation	Kidney		Lower levels	Monoclonal antibody against CD47
[35]	Microgravity	Blood vessels	Endothelial cells	Distinct pathways	
[221]	Microgravity	Blood vessels	Endothelial cells	Lower levels	
[34]	Motility	Blood vessels	Endothelial cells	Distinct pathways	
[30]	Motility	Dermis	Endothelial cells, microvascular	VEGF induces OPN	
[21]	Motility, angiogenesis, cancer	Skin	Melanoma cell line	OPN induces VEGF	Melanoma
[157]	Osteoarthritis	Bone	Osteoblasts	Higher levels	Sclerotic zones
[177]	Osteoblastic Differentiation	Bone	Bone marrow stromal cells	Higher levels	Effect of shear stress vs. no stress
[181]	Osteoblastic differentiation	Bone	Normal human osteoblasts	Higher levels	Titanium dioxide scaffolds
[176]	Osteogenesis	Bone	Liposuction-derived stem cells	Higher levels	3D scaffolds
[2]	Osteogenesis	Bone	Cord blood stem cells	Expressed	Collagen I/III scaffolds
[212]	Osteogenesis	Bone		Higher levels	Demineralized dentin matrix
[171]	Osteogenesis	Bone	Bone marrow stromal cells	Higher levels	Effect of platelet-rich plasma
[202]	Osteogenesis	Bone	Human osteoblast-like SaOS-2 cells	Higher levels	Effect of *Puerariae radix*
[200]	Osteogenesis	Bone	Osteoblasts	Distinct pathways	Electric current, biphasic
[196]	Osteogenesis	Bone	Bone marrow stromal cells	Higher levels	Flow, continuous or pulsatile
[183]	Osteogenesis	Bone	Mouse osteoblast-like cells, mouse microvascular endothelial cells	Reduced levels	Perfusion flow in 3D calcium phosphate scaffolds
[26]	Osteogenesis	Bone	Embryonic stem cells	VEGF induces OPN	Effect of BMP2 ad VEGF
[184]	Osteogenesis, angiogenesis	Bone	Bone marrow stromal cells	Higher levels	Bone defects, copper-containing mesoporous bioactive glass scaffolds
[206]	Osteogenesis/odontogenesis	Mouth	Dental pulp cells	Higher levels	Crown fracture in incisors
[82]	Osteogenic differentiation, hypoxia	Bone	Mesenchymal stem cells	Higher levels	Injury, avascular
[167]	Osteoinduction	Bone	Bone marrow stromal cells, myoblasts	Higher levels	Role of BMPs
[192]	Osteoporosis	Bone		Higher levels	Osteostatin-loaded mesoporous ceramics
[166]	Osteoporosis	Bone		Expressed	
[211]	Regeneration	Mouth	Primary osteoblasts	Upregulated	Enamel matrix derivative + bone natural mineral
[220]	Sclerosis	Abdomen		Expression correlated to MMP-2	Encapsulating peritoneal sclerosis
[43]	Skeletal disorder	Joint		Distinct expression	Calcific tendinopathy
[205]	Teeth, developing	Mouth		Expressed	
[182]	Tissue engineering	Bone	Pre-osteoblasts	Higher levels	Amorphous calcium phosphate minerals
[27]	Tissue engineering	Bone		VEGF induces OPN	Effect of BMP-2 and VEGF
[186]	Tissue engineering	Bone	Human mesenchymal stem cells	Higher levels	High hydroxyapatite:poly(lactic-co-glycolic acid) ratios

(continued on next page)

Table 1 (*continued*)

Reference	State/disease	Tissue	Cells	Induction	Condition/treatment
[187]	Tissue engineering	Bone	Preosteoblastic cells	Higher levels	Higher apatite content
[180]	Tissue engineering	Bone		Higher levels	Perforations in bone bed, iliac bone grafts
[179]	Tissue engineering	Bone		Higher levels	Perforations in receptor bed, calvarial bone grafts
[185]	Tissue engineering	Bone	Human osteoblasts	Higher OPN, no change in VEGF	Post-traumatic bone defects, demineralized bone biomatrix
[188]	Tissue engineering	Bone	Bone marrow cells	Higher levels	RAD16 + hydroxyapatite
[190]	Tissue engineering	Bone	Mesenchymal stem cells, adipose tissue-derived	Higher levels	Simvastatin coating of titanium dioxide scaffolds
[191]	Tissue engineering	Bone	Adipose tissue-derived stem cells	Higher levels	Simvastatin coating of tricalcium phosphate scaffolds
[178]	Tissue engineering	Bone	Bone marrow stromal cells	Higher levels	Stereolithography
[189]	Tissue engineering	Bone	Marrow stromal cells	Higher levels	Ti-ECM scaffolds
[199]	Tissue healing	Bone	Preosteoblastic cells	Higher levels	Thermal and tensile stress
[41]	Vascular diseases	Heart		Distinct pathways	Atherosclerosis
[155]	Vascular diseases	Kidney	Endothelial cells	Reduced levels	Diabetes
[60]	Vascular diseases	Eye	Retinal endothelial cells	Higher levels	Diabetes, retinopathy
[154]	Vascular diseases	Eye		Upregulated	Diabetic retinopathy
[153]	Vascular diseases	Lung		Higher levels	Idiopathic pulmonary fibrosis
[22]	Vascular diseases	Blood vessels	Adventitial fibroblasts	VEGF induces OPN	Injury
[23]	Vascular diseases	Blood vessels		VEGF induces OPN	Injury, vascular calcification
[42]	Vascular diseases	Heart		Distinct expression	Late pregnancy, heart hypertrophy
[13]	Vascular diseases	Eye, retina	Glial cells, retinal	OPN induces VEGF	Osmotic swelling
[156]	Vascular diseases	Bone		Distinct expression	Peripheral artery disease/treatment with ramipril
[59]	Vascular diseases	Lung		Higher levels	Pulmonary arterial hypertension
[24]	Vascular diseases	Blood vessels		VEGF induces OPN	Vascular remodeling
[67]	Vascular diseases, functional angiogenesis	Bone/skeletal tissue	Umbilical cord endothelial cells, 10T1/2, THP1, and C2C12	PGC-1α regulates OPN and VEGF expression	
[122]		Endometrium		Higher levels	Elevated progesterone levels
[73]		Bone marrow	Hematopoietic stem cells	Distinct pathways	Lipid raft clustering
[207]		Mouth	Malassez's epithelial rest cells	Higher levels	Response to stretch force
[10]		Bone/skeletal tissue	Matrix vesicles and chondrocytes	Co-expression	
[89]		Blood vessels	Endothelial cells	Independent effects	
[54]		Blood vessels	Endothelial cells	VEGF suppresses OPN	

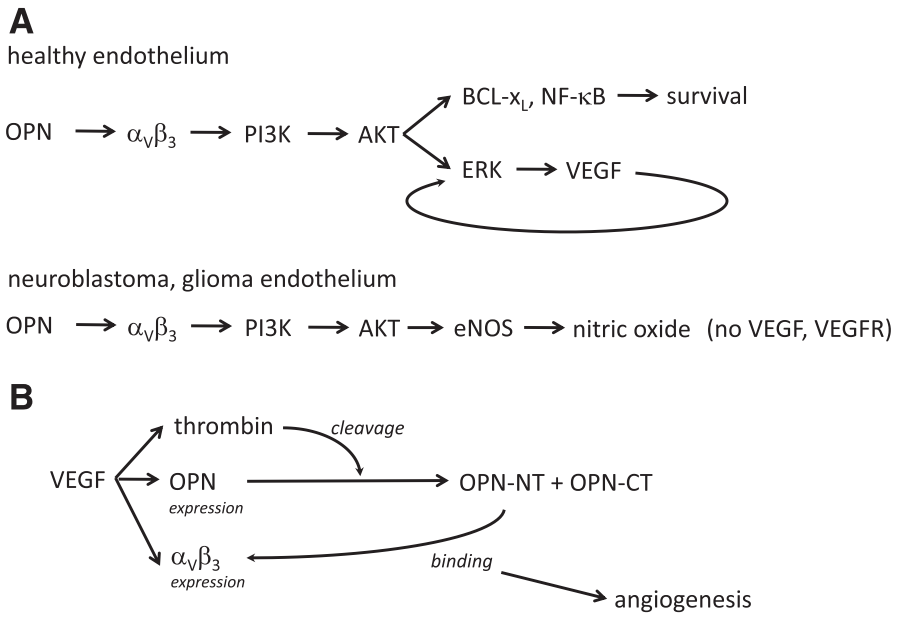

Fig. 1. Angiogenic pathways. A) OPN promotes endothelial cell motility, proliferation, and tube formation via its engagement of integrin $\alpha_v\beta_3$, which activates the PI3K/AKT pathway and confers cyto-protection through the up-regulation of BCL-X_L and NF-κB activity, while also enhancing VEGF expression. In a positive feedback loop, VEGF activates the PI3K/AKT and ERK1/2 pathways (top) [16]. By contrast, OPN secreted by glioma cells augments proliferation, migration and tube formation in endothelial progenitor cells via integrin $\alpha_v\beta_3$/PI3K/AKT signaling, thus activating eNOS and increasing NO production, independently of changes in VEGF or VEGFR expression (bottom) [37]. B) In vascular remodeling, VEGF acts as a master-regulator by coordinating the up-regulation of various pro-angiogenic target molecules, including integrin $\alpha_v\beta_3$, OPN, and thrombin. After the conversion of prothrombin to thrombin, thrombin-cleaved OPN promotes a greater rate of endothelial cell migration than native OPN [30].

[30]. In ischemia-induced retinal neovascularization, VEGF-mediated tube formation is dependent on OPN [69], suggesting that it acts through OPN induction. VEGF (also termed vascular permeability factor, VPF) mediates microvascular permeability, leading to the extravasation of blood coagulation factors and other plasma proteins. It thus activates the extrinsic coagulation pathway and the conversion of prothrombin to thrombin. Thrombin-cleaved OPN promotes a greater rate of endothelial cell migration than native OPN (Fig. 1B). Hence, the co-induction of integrin $\alpha_v\beta_3$ (the receptor involved in OPN-mediated haptotaxis/cell crawling), OPN, and thrombin by VEGF stimulates increased migratory activity by endothelial cells during angiogenesis [30].

2.1.4. Independent actions of OPN and VEGF

OPN and VEGF do not always co-express. A non-classical sonic hedgehog (SHH) signal (without the involvement of GLI family transcription factors) promotes capillary morphogenesis, endothelial cell migration and endothelial cell expression of MMP-9 and OPN via a RHO/ROCK pathway without significantly altering the VEGF levels

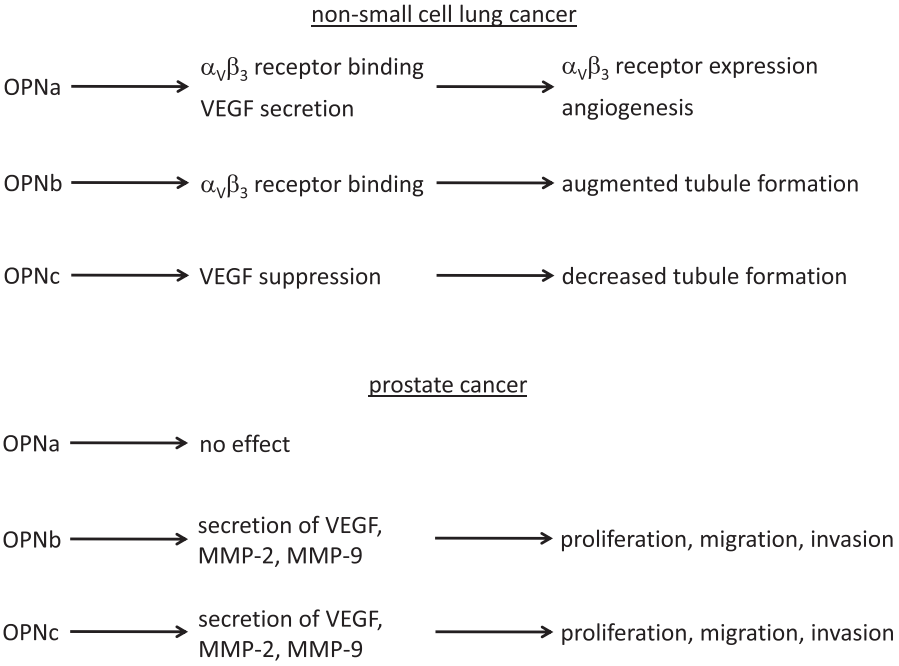

Fig. 2. OPN splice variants and VEGF in cancer. The three splice variants of OPN (full-length form OPNa, OPNb lacking exon 5, and OPNc lacking exon 4) differentially affect VEGF and angiogenesis in lung cancer. Their effects in prostate cancer are different from those in lung cancer. $\alpha_v\beta_3$ refers to the integrin receptor.

[36]. In the absence of the pro-apoptotic protein BIM in kidney endothelial cells, the expression of OPN is reduced whereas VEGF expression is elevated and correlates to increased cell migration and capillary morphogenesis without any changes in integrin-adhesion ability [46]. A lack of pigment epithelium-derived factor (PEDF) in lung endothelial cells promotes an angiogenic, cell migratory and pro-inflammatory phenotype by increasing the levels of VEGF, iNOS and VCAM-1 and by promoting the nuclear localization of β-catenin. OPN levels, however, decrease in response to PEDF deficiency [71]. Poly(methacrylic acid-co-methyl methacrylate) beads are under study as an experimental treatment to facilitate wound closure, because they stimulate a pro-angiogenic host response. Compared to poly(methyl methacrylate) beads or no treatment, these beads decrease OPN expression without affecting VEGF levels in macrophage-like cells and in endothelial cells [72].

OPN and VEGF may not always act in conjunction to promote endothelial cell motility. There are two pathways regulating endothelial cell migration, VEGF-stimulated chemotaxis is critically dependent on RAC activation, while migration induced by OPN is RAC-independent. OPN is a potent matrix activator of motility, and a likely cause for the absence of a synergistic effect is that OPN-stimulated motility is inhibitory to the RAC pathway [34]. Most hematopoietic stem cells in the bone marrow reside in a quiescent state and occasionally enter the cell cycle upon cytokine-induced activation. Lipid raft clustering is involved in hematopoietic stem cell activation, and OPN suppresses, whereas VEGF increases lipid raft clustering [73]. In contrast to the physiologic OPN-induced VEGF expression via PI3K/AKT/ERK [16], OPN secreted by glioma cells augments proliferation, migration and tube formation in endothelial progenitor cells via integrin $\alpha_v\beta_3$/PI3K/AKT signaling, thus activating eNOS and increasing NO production, independently of changes in VEGF, VEGFR-1 and VEGFR-2 expression [37]. When induced by fibroblast growth factor 2 (FGF-2) or a non-classical sonic hedgehog signal, OPN may act as a cell survival, adhesive and chemotactic factor for endothelial cells. FGF-2 causes the over-expression of a phosphorylated form of OPN, which is associated with pro-angiogenic monocyte infiltration. By contrast, VEGF is unable to increase OPN production in newly formed endothelial cells. This FGF-2-mediated, OPN-driven pro-angiogenic monocytic infiltration may be a mechanism for neovascularization during inflammation, wound healing and tumor growth [33].

Apart from the integrin $\alpha_v\beta_3$-binding RGD domain in OPN, another motif is as effective as VEGF in regulating endothelial cell adhesion and migration, as well as tube formation during angiogenesis. The sequence SVVYGLR induces cell polarity and differentiation in endothelial cells leading to an increase in adhesion and migration without affecting the proliferation rate. Its efficacy depends predominantly on the internal tyrosine residue [74,75].

2.1.5. Summary

Under conditions of hypoxia, OPN induces VEGF via HIF-1α in skeletal muscle, heart, osteogenic differentiation [57], ischemic or diabetic retina [60,69], and this connection contributes to the pathophysiology of pulmonary hypertension [58,59]. The induction of OPN and VEGF is associated with inflammation [61]. Dendritic cells enrich the stem cell pool with endothelial progenitor cells that secrete OPN and VEGF together with IL-8 and IL-10 in blood vessel formation [66]. OPN and VEGF activation attracts macrophages and induces their MCP-1 secretion [67,70].

Three main pathways of VEGF induction by OPN include the PI3K/AKT and ERK1/2 cascades that entail a positive feedback signal [16], the BRK- and NIK-mediated activation of ATF-4 [11], and the enhancement of TGF-β1 mediated signaling via SMAD3 and the kinase P38 [68]. By contrast, a non-classical sonic hedgehog (SHH) signal induces the expression of MMP-9 and OPN via a RHO/ROCK without affecting the levels of VEGF [46]. Conversely, in vascular remodeling, VEGF induces OPN together with integrin $\alpha_v\beta_3$ and thrombin. Thrombin then cleaves OPN and enhances its function [30].

The induction of VEGF without OPN causes pathologic angiogenesis in skeletal muscle. In neuroblastoma, the migration of vascular endothelial cells is associated with the induction of OPN, but not VEGF [51]. The two cytokines exert differential effects on lipid raft clustering (OPN suppresses, VEGF increases) and RAC (OPN inhibits, VEGF stimulates) [73].

2.2. Response to hypoxia

Hypoxia is a salient feature in tissue injury, ischemic diseases and solid tumors. The cellular adaptation to hypoxia is mediated through the activation of a set of specific transcription factors and genes, importantly the hetero-dimeric hypoxia inducible factor (HIF). Whereas VEGF is a direct downstream target of HIF-1α (the oxygen-sensing subunit of HIF-1), OPN is not regulated by it. However, OPN expression is enhanced under hypoxia through different mechanisms, often leading to co-expression of, and possible synergy between these cytokines.

2.2.1. Co-expression of OPN and VEGF

Hypoxia can affect cancer progression. The expression of HIF-1α, HIF-1α-inducible VEGF, and OPN in hypoxic tumors may be correlated to an aggressive phenotype. A low-oxygen environment increases the levels of OPN, HIF-1α, VEGF, and various other chemokines and pro-angiogenic mediators in myeloid leukemia cells [64,76]. In glioblastoma patients, the expression levels of the hypoxia-related markers OPN and carbonic anhydrase IX (catalyzes the reversible hydration of carbon dioxide, which regulates respiration and acid–base balance), followed by VEGF expression, are higher when compared to their expression levels in low grade astrocytomas or healthy brains under hypoxic conditions [77]. Tumor hypoxia plays a significant role in the treatment resistance of head and neck cancer. Eight hypoxia-regulated cytokines and angiogenic factors that constitute the “high-risk signature” include OPN and VEGF, along with interleukin-4, interleukin-8, growth-related oncogene-α, eotaxin, granulocyte-colony stimulating factor and stromal cell-derived factor-1α [100]. In prostate cancer patients treated surgically, an increased expression of HIF-1α, VEGF and OPN correlates to a worse outcome. In patients treated with radiotherapy only HIF-1α and VEGF, but not OPN, correlate with poor outcome [78].

Upon hypoxia-induced vascular inflammation in pulmonary hypertension, an up-regulation of VEGF and OPN occurs in the pulmonary artery adventitia before any evident accumulation of monocytes. The early induction of these factors may play an important role in the recruitment and retention of macrophages to the chronic inflammation sites [58].

During orthodontic treatment, applied forces lead to circulatory disturbances in the pressure zone of the periodontal ligament and result in hypoxic conditions. Hypoxia in cementoblasts, created by the mechanical stress, decreases cell proliferation and increases apoptosis. In addition, it time-dependently affects mineralized nodule formation. The expression pattern of OPN mRNA is correlated to the expression pattern of HIF-1α in hypoxic cementoblasts. Temporary hypoxia stimulates cementoblastic function and is associated with the elevated expression of HIF-1α, VEGF and OPN. By contrast, long-term hypoxia inhibits cementoblastic function, manifested by decreased expression of OPN, alkaline phosphatase, osteocalcin, bone sialoprotein, and osteoprotegerin [79].

2.2.2. Induction of VEGF by OPN

Under a low partial pressure of oxygen, the expression of OPN is induced in susceptible cells and is responsible for an increase in VEGF expression levels. OPN is vital for initiating VEGF-induced tube formation. The expression on proliferating vascular endothelial cells of integrin $\alpha_v\beta_3$ and its ligand OPN is elevated in the ischemic retina and contributes to VEGF-induced retinal neovascularization by stimulating interactions between endothelial cells and extracellular matrix. This effect can be inhibited by an anti-OPN antibody [69]. OPN secreted from the heart in response to myocardial ischemia causes an up-

regulation of liver-induced angiogenesis-related genes, including VEGF, which increases capillary density and improves cardiac function [14].

A hypoxia-induced HIF-1α pathway activates a pro-angiogenic phenotype in monocytic cells that infiltrate the juvenile idiopathic arthritis synovium. It leads to an induction of VEGF and OPN expression in the synovial fluid. The levels of both cytokines are sustained in a prolonged hypoxic environment but are suppressed upon re-oxygenation [80]. In mesenchymal stromal cells, the temporary exposure to hypoxia over days leads to an increase in VEGF levels through HIF-1α and a sustained up-regulation of OPN [81,82]. Hypoxia-dependent HIF-1α, VEGF and OPN enhance the expression of pro-angiogenic mediators and bone-related genes in bone tissue engineering. They may be beneficially induced with the cell-permeable prolyl hydroxylase inhibitor dimethyloxallyl glycine [83]. Hydroxylation is required for the poly-ubiquitylation and degradation of HIF-1α, whereas the non-hydroxylated form becomes stabilized and translocates to the nucleus.

Hypoxia-driven OPN induction in breast cancer cells regulates HIF-1α expression via ILK/AKT/P65 as intermediate signaling molecules, leading to VEGF activation. This results in breast tumor growth and angiogenesis [12,84]. OPN suppression in breast cancer cells decreases the levels of HIF-1α and VEGF and increases the radio-sensitivity of these cells [85].

2.2.3. Independent regulation of OPN and VEGF

The hypoxia-regulated genes OPN and VEGF may not be correlated to metastatic potential under certain conditions. The exposure of tumor cells to hypoxia, glucose starvation, or acidic pH enhances their invasive capacity in various cancer types and thus contributes to their heightened metastatic potential. However, no overall changes in the mRNA levels of metastasis-associated genes, like VEGF or OPN, were observed upon transient treatment with hypoxia, followed by a period of normoxic growth condition. This could suggest that either these genes may not have any significant role in the transient metastatic phenotype or their mRNA levels may not accurately account for the associated protein levels [86].

In bone healing, damage to the vasculature at the defect site can create a low oxygen environment that inhibits the osteogenic differentiation of adipose-derived stem cells. Hypoxia causes a HIF-1α-dependent increase in VEGF secretion in these cells, and a HIF-1α-dependent decrease in the expression of osteogenic markers, including OPN, alkaline phosphatase, and osteonectin [87].

2.3. Protection from apoptosis

OPN and VEGF have cyto-protective effects, mutually independently as well as via affecting the levels of each other. Both molecules may safeguard against programmed cell death in diverse tissues, including the mouth and transformed breast.

A loss of OPN expression in breast cancer cells decreases the levels of HIF-1α and VEGF. This contributes to increased apoptosis and radio-sensitivity as well as accelerated cancer cell senescence [85]. The treatment of breast cancer with andrographolide down-regulates OPN and VEGF. The agent increases apoptosis and inhibits the interaction between endothelial cells and tumor cells [65]. OPN confers cyto-protection to endothelial cells through the activation of the PI3K/AKT pathway with subsequent up-regulation of BCL-X_L and activation of NF-κB. It also enhances VEGF expression through the phosphorylation of AKT and ERK. The OPN-induced VEGF further activates the PI3K/AKT and ERK1/2 pathways [16].

Mechanical stress on the teeth may cause periods of hypoxia with ensuing apoptosis in cementoblasts. Temporary hypoxia stimulates cementoblastic function and is associated with the elevated expression of HIF-1α, VEGF and OPN. By contrast, long-term hypoxia inhibits cementoblastic function, manifested by a decreased expression of OPN, alkaline phosphatase, osteocalcin, bone sialoprotein, and osteoprotegerin [79]. N-Acetylcysteine (NAC) prevents the adverse effects of resin-based dental restorations by increasing cell differentiation and decreasing cell death in dental pulp stromal cells. NAC protects from a deadhesion-induced decrease in VEGF secretion and increase in apoptosis, partly by the induction and mobilization of NF-κB to the nucleus, which is then responsible for regulating various gene programs for differentiation and anti-apoptosis. The agent also increases the expression of OPN, osteocalcin and dental sialoprotein in a stepwise manner during differentiation [88].

Kidney endothelial and epithelial cells make unique contributions to the regulation of their extracellular matrix composition, with specific impact on adhesive and migratory properties that are essential for their proper function. The pro-apoptotic protein BIM (BCL2L11) contains a BH3 domain and interacts with other members of the BCL-2 family to induce cell death. Its absence in kidney epithelial cells leads to decreased migration, increased adhesion, and sustained expression of OPN and thrombospondin-1. By contrast, BIM depletion in kidney endothelial cells causes increased cell migration and capillary morphogenesis. VEGF expression is substantially increased, whereas the expression of OPN and thrombospondin-1 is reduced. The BIM-depleted endothelial cells also have decreased endothelial nitric oxide synthase activity and nitric oxide bioavailability. Their expression levels correlate to higher cell migration and capillary morphogenesis without any changes in their integrin-adhesion ability [46].

However, VEGF may negatively regulate OPN in select settings. Although VEGF alone does not affect OPN levels [54] in endothelial cells under simulated microgravity, its absence leads to higher levels of several extracellular matrix proteins and increased rates of apoptosis (in a reduced gravity environment, the extracellular matrix proteins may not be able to activate anti-anoikis signals). The presence of VEGF drastically decreases cell death and counterbalances the effect of microgravity on the expression levels of extracellular matrix proteins like OPN, collagen type I, fibronectin and laminin [54,89].

3. OPN and VEGF in disease

3.1. Cancer

OPN and VEGF are markers for poor prognosis in cancer [15,78,90–97]. Both are present in the secretome (a promising source of blood-based biomarkers) of IGF1R transformed fibroblasts [98]. OPN and VEGF contribute to tumor progression [8,12,19,20,62,99–102] and angiogenesis [11,12,16,21,28,37,93,99,100,103–108]. The inhibition of OPN may reduce VEGF levels and suppress tumor progression [85,109–112].

3.1.1. Lung cancer and mesothelioma

In various subtypes of pulmonary malignancies, OPN and VEGF contribute variably to progression. In adenocarcinoma, they jointly support intra-tumoral micro-vessel density, angiogenic and metastatic activity, and in rare cases pulmonary tumor thrombotic micro-angiopathy. In non-small cell lung cancer, they predict poor prognosis. The three OPN splice variants interact differentially with VEGF, with the full-length form OPNa enhancing VEGF secretion and the shortest form OPNc reducing it.

Elevated OPN blood concentrations in non-small cell lung cancer are associated with worse disease prognosis and shorter patient survival [92]. The analysis of public databases has identified a select number of genes, including VEGF and OPN, to be biomarkers for poor survival of non-small cell lung cancer patients [90]. The expression of OPN and VEGF is correlated with intra-tumoral micro-vessel density in patients with lung adenocarcinoma, but not in patients with lung squamous cell carcinoma. The cooperation with OPN is important for VEGF-mediated, integrin $\alpha_v\beta_3$-dependent angiogenesis and metastasis, as VEGF alone only weakly influences the prognosis of patients with the disease. The presence of OPN along with VEGF increases both the angiogenic as well as the metastatic activity of lung adenocarcinoma, and

confers to patients a worse prognosis than the expression of neither or only one of these biomolecules [62]. In very rare cases, lung adenocarcinoma causes pulmonary tumor thrombotic micro-angiopathy, where OPN and VEGF together promote the intimal proliferation in pulmonary arteries and arterioles [7]. During advanced stage lung adenocarcinoma, VEGF secretion by mesothelial and endothelial cells, stimulated by OPN, is increased in malignant pleural effusion and forms new blood vessels. Host- and tumor-derived OPN exert differential effects in this setting. Host OPN regulates tumor-associated angiogenesis and inflammation, whereas tumor OPN has cancer cell survival benefits during malignant pleural effusion pathogenesis [15,104].

The three splice forms of OPN have divergent roles on VEGF secretion and tubule formation in non-small cell lung cancer pathogenesis. The full-length protein OPNa not only increases tubule formation via its action on the receptor integrin $\alpha_v\beta_3$ but also enhances the secretion of VEGF by tumor cells [18], which in turn increases the expression of integrin $\alpha_v\beta_3$ receptors on endothelial cells [30]. The splice form OPNb augments tubule formation via integrin $\alpha_v\beta_3$ but does not affect VEGF expression. OPNc, on the other hand, has a dominant negative effect and causes a decrease in VEGF expression in non-small cell lung cancer and decreased tubule formation [18].

Multiple-marker panels may inform on the disease state in lung cancer. In non-small cell lung cancer, tenascin-c, which is physiologically important during fetal development, predicts poor survival and correlates with micro-vessel density, OPN, and VEGF, suggesting that it may exert its effect via angiogenesis, mediated by VEGF and OPN [8]. A combination of VEGF and OPN together with carbonic anhydrase IX is additively correlated to prognosis in M0-stage non-small cell lung cancer, and these biomarkers together are able to predict the overall post-radiotherapy survival in these patients [91]. Down-regulation of the putative tumor suppressor gene TIP30 (HTATIP2) elevates the levels of OPN and VEGF, along with MMP-2, thereby enhancing anchorage-independent cell growth, cancer cell survival, lung cancer angiogenesis and metastasis [113] (Table 2A).

OPN and VEGF have been targets for anti-lung cancer therapies. Ethanol extracts of ocimum sanctum inhibit OPN-mediated cell adhesion and invasion in non-small cell lung cancer. They effectively attenuate the expression of OPN and CD44, as well as the OPN activated expression of CD44. Under treatment, the expression of urokinase plasminogen activator (uPA), its receptor uPAR, and the epidermal growth factor receptor are attenuated at the mRNA level and the production of VEGF and activity of MMP-9 are reduced [114]. The experimental anti-cancer drug tirapazamine is selectively activated by multiple reductases to form free radicals in hypoxic cells, thereby inducing DNA damage and cell death. A clinical trial using tirapazamine in addition to carboplatin and paclitaxel analyzed the relationship between clinical outcomes and plasma levels of hypoxia-associated OPN along with VEGF and plasminogen activator inhibitor-1 (PAI-1). As non-small cell lung cancer patients with lower OPN levels have a better outcome regardless of the presence of tirapazamine in the treatment, there is no additional benefit of the drug. Although the levels of both VEGF and PAI-1 declined after therapy, neither of these markers could be correlated to outcome [92]. In lung cancer, OPN increases VEGF protein levels, which leads to angiogenesis. The anti-epileptic drug topiramate may exert anti-tumor and anti-metastatic effects on lung cancer by decreasing OPN levels, and thus the growth of small blood vessels, especially around the topiramate-treated area [108] (Table 2B).

OPN and VEGF are present in abundance in mesothelioma. OPN levels are higher in patients with mesothelioma, of both epithelioid and sacromatoid subtypes, than in individuals with asbestos-related, non-malignant pleural disease, although they directly correlate with the duration of the asbestos exposure. VEGF levels are higher in mesothelioma patients than in any other solid tumor, and therapeutic agents in clinical trials for this disease consistently include inhibitors of VEGF or VEGF receptors [115].

3.1.2. Breast cancer

There is a close association between VEGF expression and OPN expression in breast cancer. OPN regulates VEGF expression by tumor cells and also has a substantial role in VEGF-dependent neovascularization. Therefore, the suppression of OPN leads to a reduction of VEGF levels in the tumor [85,109]. The interaction between OPN and its receptor integrin $\alpha_v\beta_3$ on breast cancer cells activates pathways involving breast tumor kinase (BRK)/nuclear factor-inducing kinase (NIK)/NF-κB/ATF-4 through autocrine and paracrine mechanisms to promote tumor cell migration, tumor growth, endothelial migration, and angiogenesis. Hypoxia-driven OPN induction in breast cancer cells regulates HIF-1α expression via ILK/AKT/P65 as intermediate signaling molecules, prompting VEGF activation and leading to breast tumor growth and angiogenesis [11,12] (Fig. 3).

Lymph node and bone marrow metastases are inversely correlated to each other for early stage, localized breast cancers, suggesting independent pathways for lymphatic and hematogenous tumor dissemination. OPN and VEGF expression are variably associated with metastases in these sites. Higher OPN and CD54 expression is positively correlated with bone marrow metastasis and negatively correlated with lymph node metastasis. VEGF-C, on the other hand, is up-regulated in both lymph node metastatic and bone marrow metastatic tumors without reaching the levels of statistical significance in the lymphatic vs. the hematogenous pathway comparison [40].

OPN and VEGF may be targets for anti-cancer treatment. Curcumin inhibits breast cancer angiogenesis. It down-regulates OPN-induced

Table 2
OPN and VEGF in lung cancer. A) Marker combinations in lung cancer. Select molecules have been studied as lung cancer biomarkers in conjunction with OPN and VEGF. B) Experimental lung cancer treatment that targets OPN and VEGF.

A

Marker	Description	Function
Tenascin-c	Physiologically important during fetal development	Predicts poor survival and correlates with micro-vessel density, OPN, and VEGF
Carbonic anhydrase IX	Regulates respiration and acid–base balance	Combination with VEGF, OPN is correlated to M0-stage prognosis and predicts overall post-radiotherapy survival
TIP30	Putative tumor suppressor gene	Down-regulation elevates OPN, VEGF, MMP-2, enhancing anchorage-independence, angiogenesis, metastasis

B

Cancer	Drug	Outcome
Non-small cell lung cancer	Ethanol extracts of ocimum sanctum	Inhibition of cell adhesion and invasion; attenuated expression of OPN and CD44, and OPN-activated CD44 expression; reduced production of MMP-9 and VEGF
Non-small cell lung cancer	Tirapazamine in addition to carboplatin and paclitaxel	Low OPN predicts better outcome (regardless of the presence of tirapazamine), declining levels of VEGF and PAI-1 cannot be correlated to outcome; there is no additional benefit of the drug
Lung cancer	Topiramate	May exert anti-tumor and anti-metastatic effects by decreasing OPN levels, thus the growth of small blood vessels

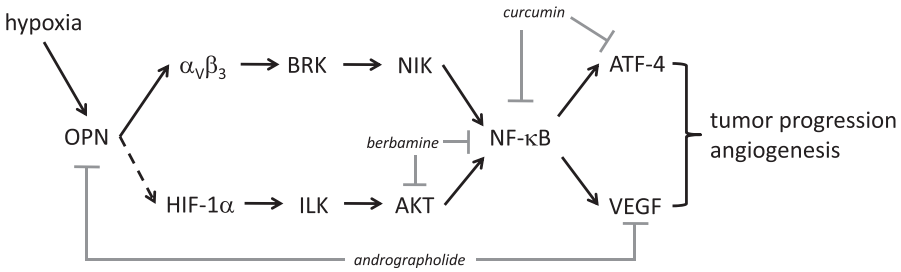

Fig. 3. Signaling pathways in breast cancer. OPN promotes tumor progression and angiogenesis through two cross-communicating pathways. Both involve the activation of the transcription factor NF-κB and the induction of specific genetic programs. These pathways are targets for several candidate anti-cancer drugs (shown in italics, see also Fig. 4).

VEGF expression in breast cancer by preventing the activation of the transcription factors NF-κB and ATF-4. The agent also suppresses OPN-induced VEGF/NRP-1-dependent breast tumor motility and this effect is enhanced in the presence of an anti-VEGF or anti-NRP-1 antibody in comparison to curcumin alone [112]. Andrographolide down-regulates the expression of both OPN and VEGF along with COX-2. OPN inhibition decreases AKT phosphorylation in a c-JUN-dependent manner in breast cancer cells, thus reducing cell proliferation, cell migration, angiogenesis, and tumor growth. Andrographolide increases apoptosis and inhibits the interaction between endothelial cells and tumor cells [65]. The alkaloid berbamine time- and dose-dependently suppresses growth, tumor angiogenesis, migration and invasion of highly metastatic breast cancer cells. It exerts its effect by suppressing NF-κB expression and by reducing the phosphorylation of the receptor tyrosine kinase c-MET and the signaling kinase AKT, thus reducing the expression of their downstream targets including OPN and VEGF [116] (Fig. 4).

OPN knock-down decreases the levels of HIF-1α and VEGF and increases the radio-sensitivity of these cells [85]. Blockade of OPN binding to its cell surface receptors (CD44 and integrin $\alpha_v\beta_3$) using an OPN-directed RNA aptamer leads to a decrease in the expression of VEGF, interleukin-10, PDGF and anti-apoptotic genes, while up-regulating GM-CSF, anti-proliferative, anti-metastatic and pro-apoptotic genes [109]. A gene therapy approach against OPN, utilizing siRNA delivered with a poly(amino ester) based on glycerol propoxylate triacrylate and spermine, inhibits tumor growth along with the suppression of the OPN-dependent proteins VEGF, FGF-2, cyclooxygenase-2, CD31 and MMP-9. It thereby reduces angiogenesis and invasion of tumor cells [117].

3.1.3. Gynecologic cancers

For an effective treatment of ovarian cancer, early detection is important, which is mostly done by the analysis of CA125 levels in the blood. For many cases, however, CA125 may be weakly present or absent at the initial disease stage. Both OPN and VEGF can serve as early detection markers for ovarian cancer, individually as well as together. OPN is present in all cases of ovarian cancer with low or absent CA125 along with kallikrein 10, kallikrein 6 and claudin 3. OPN, although up-regulated, does not have sufficient specificity by itself. VEGF is also expressed but only in a smaller fraction of those patients. A combination of elevated levels of VEGF and claudin 3 is able to distinguish ovarian cancers from normal ovarian surface epithelial cells. In addition, VEGF may serve as a therapeutic target [118–121].

OPN and VEGF are elevated in the endometrium in response to progesterone [122]. The expression levels of VEGF and OPN have a positive correlation to each other in cervical carcinoma. Out of close to 100 genes up-regulated in cervical squamous cell carcinoma compared to normal cervical epithelium, the gene encoding for OPN is over-expressed by 10-fold. Also, VEGF is over-expressed in cancer tissue by about 75% [123].

3.1.4. Prostate and urinary tract cancers

The preoperative levels of OPN and VEGF were not found to be significantly different between prostate cancer patients with and without lymph node metastasis [124]. Nevertheless, for patients with localized prostate cancer, the co-expression of HIF-1α, HIF-1α-inducible VEGF, and OPN is correlated to an aggressive tumor phenotype and worse treatment outcome [78], suggesting that OPN and VEGF are early markers of invasiveness with lesser value at later stages. The transcription factor RUNX2, which is associated with osteolytic disease, increases the expression of both VEGF and OPN in metastatic prostate cancer along with MMP-9 and -13, parathyroid hormone-related protein (PTHrP) and interleukin-8, which jointly enhance prostate cancer progression and the accompanying metastatic bone disease [125]. Expression of the metastasis suppressor gene gravin may suppress

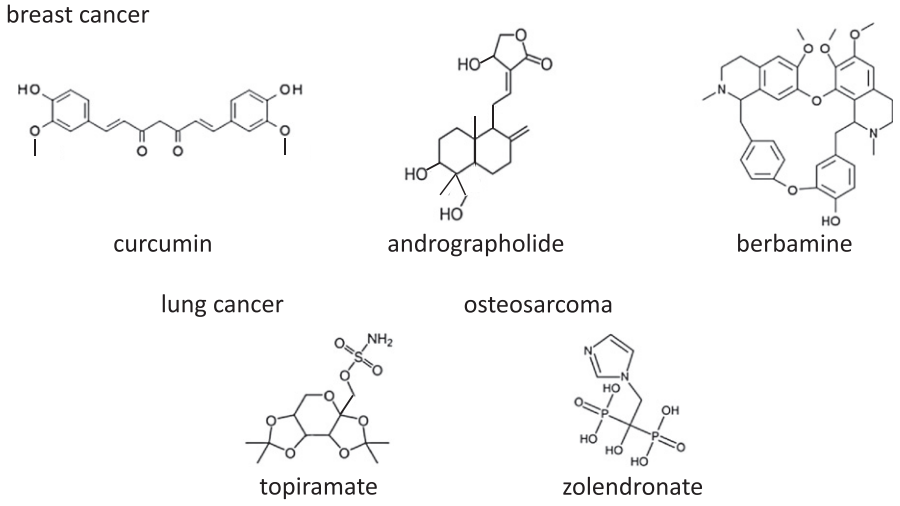

Fig. 4. Drugs that target OPN and VEGF in cancer. Various agents may suppress the secretion of function of both OPN and VEGF in specific cancers. Their actions are typically indirect.

intracellular and secreted VEGF levels in both tumor and stromal cells. VEGF inhibition, possibly via a decrease in SRC activity, leads to a down-regulation of pro-angiogenic genes including OPN, angiopoietin, HIF-1α, platelet-derived growth factor receptor β, tenascin C, and keratinocyte growth factor and to an up-regulation of anti-angiogenic genes like vasostatin and collagen 18α1 [126]. Prostate tumor endothelial cells have multi-potent plasticity. Some canonical endothelial cell markers, including VEGFR-2, CD31 and von Willebrand Factor, are expressed whereas others, such as CD146, are absent. The tumor endothelial cells undergo a mesenchymal-transition to form both cartilage- and bone-like tissues. OPN is up-regulated along with osteocalcin and alkaline phosphatase during osteogenic differentiation while collagen 2α1 and SOX9 are expressed via chondrogenic differentiation [57].

Urothelial cell carcinoma contains higher VEGF-A and OPN transcript levels than the healthy urothelium. Higher VEGF-A and OPN levels also arise in muscle-invasive, compared to non-muscle-invasive bladder cancer and are associated with poor disease-specific survival [94].

Whereas VEGF is a potent biomarker for the prediction of post-surgical metastasis and survival in patients with clear cell renal cell carcinoma, the role of OPN as a prognostic for metastasis and survival in these patients has been less well defined. To predict the pharmacotherapy responsiveness of this carcinoma, both OPN and VEGF, along with VEGF receptors (VEGFR-2 and VEGFR-3), may serve as biomarkers to determine the likely efficacy of drugs, such as pazopanib and sunitinib. Together, OPN and VEGF can predict progression-free survival in patients with metastatic renal cell carcinoma when treated with sorafenib alone or a combination of sorafenib and IFN-α [127,128].

3.1.5. Gastrointestinal cancers

OPN and VEGF, along with COX-2, are over-expressed in gastric cancer and have pro-angiogenic functions. Although they are not individually related to prognosis, their co-expression correlates with stage, lymph node metastasis, distant metastasis and micro-vessel density [99,100]. VEGF is important in the induction of gastrointestinal inflammation and cancers via its role in the up-regulation of the nuclear receptor NR4A2, which is a downstream target in the COX-2 pathway. NR4A2 subsequently activates OPN by binding directly to the NR4A2 response element in the OPN promoter (Fig. 5). Thus, VEGF induction in gastrointestinal cancers leads to angiogenesis, invasion and metastasis via OPN induction through NR4A2 [31]. COX-2 in gastric cancer may also impact VEGF, because its down-regulation can reduce the levels of VEGF and OPN and suppress tumorigenicity and angiogenesis [129]. A mechanism by which OPN may support tumor progression is via up-regulation of VEGF and induction of angiogenesis. Thus, inhibition of OPN by siRNA suppresses both OPN and VEGF expression and leads to a decrease in gastric tumor growth and its metastasis [110]. However, in a contrasting study, inhibition of OPN expression by siRNA knockdown in gastric cancer cells reduced the proliferation, migration and tube formation ability of endothelial cells and micro-vessel density, independently of VEGF expression [50].

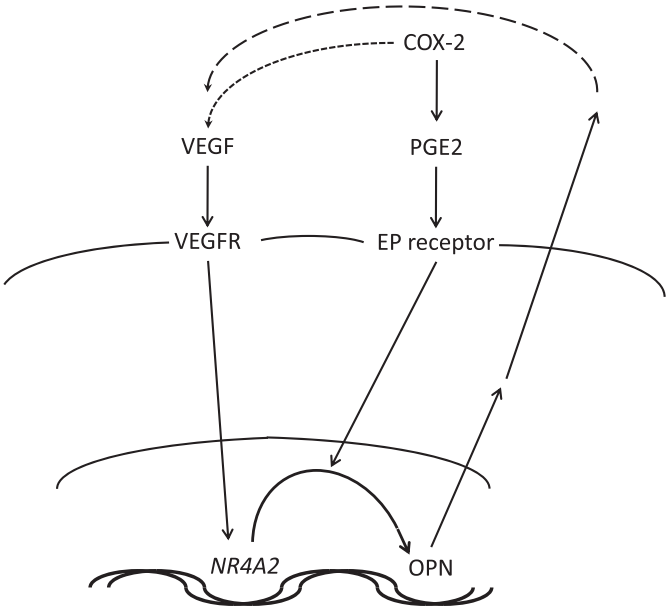

Fig. 5. OPN, VEGF, and COX2 in gastric cancer. Proposed pathway, through which VEGF and prostaglandin E_2 (PGE2) may induce OPN expression in gastric cancer cells. NR4A2 is a transcription factor, COX-2 denotes the enzyme cyclooxygenase 2. A report that COX-2 down-regulation may suppress VEGF implies a direct interaction (dotted arrow). The influence of OPN on VEGF expression (dashed arrow) completes a positive feedback loop.

In pulmonary tumor thrombotic micro-angiopathy, induced by gastric adenocarcinoma with severe hypertension, tumor cells in both the primary and pulmonary lesions express OPN and VEGF along with platelet-derived growth factor. Also, these proteins are expressed by fibromuscular intimal cells that undergo proliferation [63]. OPN and VEGF mediate the narrowing of the pulmonary arteries due to fibrocellular intimal proliferation and thrombus formation [63,130,131]. In a separate study of pulmonary tumor thrombotic micro-angiopathy induced by gastric carcinoma metastasis, patients displayed elevated tissue factor reactivity, while the correlation of VEGF and OPN with the disease was considered uncertain [131].

Obesity is an important risk factor for colorectal cancer. The gene expression levels of various angiogenesis-related molecules (VEGF, HIF-1α, MMP-2), pro-inflammatory adipocytokines and inflammatory markers (lipocalcin, OPN, TNF-α, chitinase-3 like-1, interleukin-6, hepatocyte growth factor) are elevated in the visceral adipose tissue of patients with colorectal cancer [132]. Increased levels of VEGF and TIMP-1, but not OPN, are positively correlated with metachronous and synchronous liver metastasis and intrahepatic recurrence following resection [47]. Irradiation of colorectal adenocarcinoma induces the expression of VEGF, its receptor VEGFR2 and OPN, thus intensifying the failure of radiation therapy by stimulating vascular regrowth. Therefore, anti-angiogenic therapies and radiation therapy require a post-treatment evaluation of the VEGF/VEGFR2 pathway either directly or indirectly by measuring OPN. The latter serves as a surrogate marker for VEGF/VEGFR2 because the two important angiogenic factors VEGF and OPN are co-expressed and are directly correlated to tumor proliferation [5]. OPN knockdown may lead to a decrease in colon cancer cell growth and invasion and a subsequent reduction in the expression of the angiogenic factor VEGF [111]. One contrasting study found OPN to be inversely correlated to VEGF expression in colorectal cancer [133].

3.1.6. Liver and pancreatic cancers

Detection of hepatocellular carcinoma at an early stage is a prerequisite for a favorable treatment prospect. OPN and VEGF, in combination with other markers, may be suitable for early diagnosis and for predicting a poor prognosis. Adhesion molecules and angiogenic factors, along with proteinases and other molecules, play important roles in hepatocellular carcinoma invasion and metastasis. In a signature of about 150 genes that characterize progression and recurrence of this cancer, OPN is a lead gene, which exerts its effect on tumor progression through mediating cancer–stromal interactions. It is associated with intrahepatic metastasis and early recurrence. VEGF is an independent predictor of progression, as it is associated with venous invasion, advanced tumors, and metastatic recurrence [93,103,105]. Among six proteins with corroborated importance in hepato-carcinogenesis (E-cadherin, ICAM, MMP-2, β-catenin, OPN, and VEGF), β-catenin is a potential early marker for hepatitis C virus (HCV)-associated hepatocellular carcinoma, the levels of OPN are significantly elevated in patients with hepatocellular carcinoma and chronic hepatitis over asymptomatic HCV genotype 4 carriers or healthy controls. On the other hand, VEGF levels are significant

in predicting disease progression from liver cirrhosis to hepatocellular carcinoma [134]. PARP-1 inhibition in hepatocellular carcinoma limits tumor growth and vasculogenesis by decreasing the levels of OPN, VEGF, interleukin-6, interleukin-1β, and tumor necrosis factor and by suppressing proliferation and NF-κB activation [135]. Combined gene therapy with interferon-αI and SG600-IL-24 (adenovirus-mediated interleukin-24 expression) is an effective means for anti-tumor activity through down-regulating the levels of both OPN and VEGF [136].

Very aggressive pancreatic cancers undergo peritoneal dissemination and hematogenous metastasis to the liver. OPN and VEGF are present in high abundance [119]. Sub-lines of pancreatic cancer cells, which metastasize to the liver or disseminate to the peritoneum, display differential gene expression. An up-regulation of angiogenic and adhesive molecules, VEGF and OPN, along with hepatocyte growth factor (HGF) characterizes the highly liver metastatic cells [102]. Cigarette smokers have an elevated risk of pancreatic ductal adenocarcinoma progression. Nicotine mediates its metastatic and pro-angiogenic effects through the downstream effector OPN. The splice variant OPNc, which is specifically expressed in cancer tissues, is induced by nicotine and upregulates the expression of MMP-9 and VEGF. Therefore, VEGF, OPN and MMP-9 co-localize in the malignant ducts of pancreatic adenocarcinoma and their high levels correlate with an invasive phenotype [19]. OPN and VEGF are expressed in osteoclast-like giant cell tumors while cystadenocarcinomas lack both these molecules. Hence, the expression of OPN and VEGF differentiates osteoclast-like giant cell tumors with undifferentiated carcinoma from osteoclast-like giant cell tumors with mucinous cystadenocarcinoma for the prediction of clinical outcome in patients with this rare form of pancreatic neoplasms [137].

3.1.7. Brain tumors

In malignant glioblastoma, VEGF levels are positively correlated with the levels of thrombin-cleaved and thrombin/carboxypeptidase-double cleaved OPN [138]. The co-localization of OPN, its receptor integrin $\alpha_v\beta_3$ and VEGF serves as a marker for glioma angiogenesis [28] and can present as a target for anti-angiogenic therapies [29]. Tumor-derived OPN does not increase VEGF production and does not affect the gene expression of other angiogenic factors in neuroblastoma cells, although OPN induces the migration of vascular endothelial cells, resulting in angiogenesis [51]. However, VEGF induces the expression of OPN along with its receptor integrin $\alpha_v\beta_3$ [28,30]. Both OPN and VEGF, in conjunction with carbonic anhydrase IX, are regulated by hypoxia. In glioblastoma patients under low oxygen conditions, the expression of OPN and carbonic anhydrase IX is the strongest, followed by VEGF levels, compared to their levels in low grade astrocytoma or in healthy brains [77]. True ossification within benign brain tumors is rare. Both VEGF and OPN are expressed in ossified choroid plexus papilloma during osteogenesis in benign brain tumors [139].

3.1.8. Head and neck cancer

OPN, along with interleukin-4 and interleukin-6, is a primary diagnostic marker in head and neck squamous cell carcinoma patients, whereas VEGF levels do not significantly differentiate between cancer patients and healthy persons [140]. Tumor hypoxia plays a significant role in the treatment resistance of head and neck cancer. Eight hypoxia-regulated cytokine and angiogenic factors, that constitute the "high-risk signature" include OPN and VEGF, together with interleukin-4, interleukin-8, growth-related oncogene-a, eotaxin, granulocyte-colony stimulating factor and stromal cell-derived factor-1α [101]. In advanced head-and-neck cancer patients treated with radiotherapy or combined chemo-radiation, high OPN and VEGF levels are correlated to HIF-1α expression [141] and tissue OPN levels are correlated to high blood VEGF levels but the prognostic value of tissue OPN alone is limited as its levels are significantly correlated with median oxygen pressure only in stage IV patients [6]. Elevated levels of OPN and VEGF mRNA and proteins arise in the epithelial and sub-epithelial areas of sino-nasal inverted papilloma tissues and have a direct correlation with tumor stage, suggesting that they contribute to tumor progression. Whether OPN directly affects the levels of VEGF in this setting remains to be elucidated [142].

3.1.9. Melanoma

OPN and VEGF are over-expressed in most melanoma cell lines [143]. In addition, stroma-derived OPN plays a crucial role in melanoma progression. In its absence, growth, anogiogenesis and metastasis are reduced. Furthermore, this stroma-derived OPN induces VEGF, the export transporter ABCG2, and the signaling intermediates ERK1/2 along with regulating the stem cell micro-environment [21]. The suppression of OPN and VEGF levels inhibits melanoma progression. For instance, the extract from lang-du roots (a remedy from traditional Chinese medicine) exerts its tumor suppressive effect via down-regulating VEGF and OPN levels [144].

3.1.10. Osteosarcoma

The roles of OPN and VEGF in osteosarcoma are complex because of their diverse roles in bone remodeling and carcinogenesis. While the levels of OPN and VEGF correlate with each other in osteosarcoma, there is no significant connection between their expression and patient outcome [3]. The interactions between cancer cells, their micro-environment and the host play an important role in tumor growth and the metastatic cascade. The levels of OPN, bone sialoprotein and decorin are higher in less aggressive than in more aggressive osteosarcoma, suggesting greater bone differentiation by the less aggressive cells. Although angiogenesis is higher in the more aggressive forms of osteosarcoma, the expression levels of angiogenesis genes including FLT1, FLT4, TIE1, TIE2 and VEGF are not significantly elevated [145]. In a patient with rare primary breast osteosarcoma, the levels of both OPN and VEGF were elevated along with the expression of various other osteoblastic and chondroblastic markers. These tumor cells showed formation of osteoid, osseous and cartilaginous intracellular substances [4]. The administration of zoledronic acid may reduce malignancy by stimulating the differentiation of cancer cells. The drug decreases the levels of alkaline phosphatase, increases the levels of osteocalcin, and transiently increases OPN over hours but decreases it to baseline within days. These target molecules serve as the early, mid-phase and late markers of bone maturation, respectively. Zoledronic acid also suppresses the expression levels of VEGF mRNA by approximately 3-fold and limits osteosarcoma growth [146].

3.1.11. Embryonic tumors

In a very rare and uncommon case of recurrent multiple chorioangioma, the expression levels of VEGF were decreased whereas those of OPN were increased. However, due to the complex nature of gene expression, no single candidate gene has been implicated as a marker for this disease [49].

3.1.12. Hematologic malignancies

OPN may serve as a useful clinical marker in patients with multiple myeloma. It is an indicator of progression, the blood levels of which correlate positively with bone destruction and tumor burden and, along with the angiogenic marker VEGF, with the micro-vessel density in the bone marrow. Both OPN and VEGF blood levels are significantly higher in stage III over stages I and II [96,97]. Multiple myeloma expansion involves bone destruction via osteoclastogenesis, which is induced by transformed cells. There is abundant interaction between myeloma cells and osteoclasts, thus that OPN (secreted by osteoclasts) in concert with VEGF (secreted by multiple myeloma cells) stimulates the migration and survival of endothelial cells and increases vascular tubule formation and angiogenesis, which promotes disease progression [106,147,148]. A blockade of VEGF and OPN may be beneficial as it inhibits endothelial cell survival, migration and vascular tubule formation [106]. Therapy that targets myeloma cells and their micro-environment

leads to a decrease in angiogenic cytokines (VEGF, fibroblast growth factor 2, angiopoietin-1, angiopoietin-2), osteoclast regulators (OPN, osteoprotegerin), dickkpof-1 and bone resorption. The VMDT regimen, constituting the administration of bortezomib (Velcade), melphalan, dexamethasone and intermittent thalidomide, achieves a reduction in myeloma-associated angiogenesis and abnormal bone remodeling with an overall response rate of around 2/3 [149].

A hypoxic environment (1% O_2) increases the levels of OPN, VEGF and various other chemokines and pro-angiogenic mediators in myeloid leukemia cells due to the increase in HIF-1α expression [64].

The interaction between stromal fibroblasts and chronic lymphocytic leukemia (CLL) cells affects the balance between pro-angiogenic and anti-angiogenic molecules to stimulate neo-angiogenesis. Specifically, this interaction up-regulates VEGF and OPN expression while inhibiting the anti-angiogenic thrombospondin-1 [107].

OPN and VEGF expression levels correlate well with occurrence and angiogenesis in acute leukemia. Their levels in the blood are significantly higher in de novo, unremitted or relapsed patients than in completely remitted patients or healthy controls [95].

3.2. Anti-cancer drug treatment

The inhibition of OPN can reduce VEGF levels and suppress tumor progression (Table 3). A blockade of VEGF and OPN may be beneficial as it inhibits endothelial cell survival, migration and vascular tubule formation [106]. Via direct action, a bispecific antibody to VEGF and OPN is capable of inhibiting tumor growth and metastasis to the lungs [150]. Via indirect action, the transcription factor NF-κB may be an upstream target for the induction of these molecules, to be inhibited by curcumin [112], berbamine [116], or PARP-1 inhibitors [135].

An extract from lang-du roots (a remedy from traditional Chinese medicine) exerts its tumor suppressive effect on melanoma progression via down-regulating VEGF and OPN levels [144]. Andrographolide down-regulates the expression of both OPN and VEGF along with COX-2 in breast cancer. It increases apoptosis and inhibits the interaction between endothelial cells and tumor cells [65]. In breast cancer, curcumin inhibits OPN-induced VEGF expression. It also suppresses OPN-induced VEGF/NRP-1-dependent breast tumor motility. Further, curcumin inhibits breast cancer angiogenesis. This effect is enhanced in the presence of an anti-VEGF or anti-NRP-1 antibody in comparison to curcumin alone [112]. The alkaloid berbamine time- and dose-dependently suppresses growth, angiogenesis, migration and invasion of highly metastatic breast cancer cells. It exerts its effect by suppressing signaling that would otherwise activate the expression of the downstream targets OPN and VEGF [116]. PARP-1 inhibition in hepatocellular carcinoma limits tumor growth and vasculogenesis by decreasing the levels of OPN, VEGF, interleukin-6, interleukin-1β, and tumor necrosis factor and by inhibiting proliferation and NF-κB activation [135]. The anti-epileptic drug topiramate may exert anti-tumor and anti-metastatic effects on lung cancer by decreasing OPN and VEGF levels, thus suppressing the growth of small blood vessels, especially around the topiramate-treated area [108]. The administration of zoledronic acid may reduce malignancy by stimulating the differentiation of cancer cells. The drug lowers the levels of alkaline phosphatase, increases osteocalcin, and transiently increases OPN over hours (but decreases it to baseline within days). Zoledronic acid also decreases the expression levels of VEGF mRNA by approximately 3-fold and suppresses osteosarcoma growth [146].

Molecularly targeted drugs may affect the levels of OPN and VEGF in cancer. To predict pharmacotherapy responsiveness in patients with clear cell renal cell carcinoma, OPN and VEGF, along with VEGF receptors -2 and -3, can serve as biomarkers for the likely efficacy of kinase inhibitors, including pazopanib and sunitinib. A systemic, tumor-independent multi-organ response to sunitinib leads to increased levels of VEGF and OPN in the blood, along with SDF-1α, SCF, G-CSF, and sTIE-2 [151]. Endoglin is a type I membrane glycoprotein located on cell surfaces that constitutes part of the TGF-β receptor complex. It is over-expressed on rapidly proliferating cancer cells. An antibody against endoglin increases the levels of OPN and VEGF-A, while decreasing VEGF-D levels over pre-treatment baseline levels [152].

Combination chemotherapy may affect multiple elements of carcinogenesis. The experimental anti-cancer drug tirapazamine is selectively activated by multiple reductases to form free radicals in hypoxic cells, thereby inducing DNA damage and cell death. In a clinical trial that added tirapazamine to carboplatin and paclitaxel for non-small cell lung cancer, patients with lower OPN levels had a better outcome regardless of the presence of tirapazamine in the treatment. Hence, there was no additional benefit of the drug [92]. Therapy that targets myeloma cells and their micro-environment leads to a decrease in angiogenic cytokines (VEGF, fibroblast growth factor 2, angiopoietin-1, angiopoietin-2), osteoclast regulators (OPN, osteoprotegerin), and dickkpof-1. Thus, it causes bone resorption. The VMDT regimen, constituting the administration of bortezomib (Velcade), melphalan, dexamethasone and intermittent thalidomide, achieves a significant reduction in myeloma-associated angiogenesis and abnormal bone remodeling with an overall response rate of around 2/3 [149].

Molecular strategies are under study as future treatment regimens. In breast cancer, blockade of OPN binding to its cell surface receptors (CD44 and integrin $\alpha_v\beta_3$) with an OPN-directed RNA aptamer leads to a decrease in the expression of VEGF, interleukin-10, platelet-derived growth factor and anti-apoptotic genes, while up-regulating GM-CSF, anti-proliferative, anti-metastatic and pro-apoptotic genes [109]. OPN knock-down in colon cancer cells may lead to a decrease in growth and invasion, and a subsequent reduction in VEGF expression [111]. OPN knock-down decreases the levels of HIF-1α and VEGF and increases the radio-sensitivity of breast cancer cells [85]. OPN-directed siRNA, delivered with a poly(amino ester) based on glycerol propoxylate triacrylate and spermine, inhibits tumor growth, invasion, and angiogenesis along with the suppression of the OPN-dependent proteins VEGF, FGF-2, cyclooxygenase-2, CD31 and MMP-9 [117]. Inhibition of OPN by siRNA suppresses both OPN and VEGF expression and leads to a decrease in gastric tumor growth and its metastasis [110]. However, in a contrasting study, inhibition of OPN expression by siRNA knockdown in gastric cancer cells reduced the proliferation, migration and tube formation ability of endothelial cells and micro-vessel density, independently of VEGF expression [50]. Combined gene therapy with interferon-αl and SG600-IL-24 (adenovirus-mediated interleukin-24 expression) is an effective means for anti-tumor activity through suppressing the levels of both OPN and VEGF [136].

OPN and VEGF are markers for therapy success in radiation treatment. In advanced head-and-neck cancer patients treated with radiotherapy or combined chemo-radiation, high OPN and VEGF may be reflective of tissue oxygen pressure [141,6]. Irradiation of colorectal adenocarcinoma induces the expression of VEGF, VEGFR2 and OPN, thus intensifying the failure of radiation therapy by stimulating vascular regrowth. Post-treatment evaluation of relevant pathways is important [5].

3.3. Vascular diseases

3.3.1. Expression of OPN and VEGF

Both OPN and VEGF may contribute to the narrowing of arteries in various pathological conditions. Together, they can promote the intimal proliferation in pulmonary arteries and arterioles and the development of pulmonary tumor thrombotic micro-angiopathy. In metastatic gastric carcinoma or rare cases of lung adenocarcinoma, up-regulated VEGF and OPN expression in primary tumor cells and subsequently in pulmonary micro-angiopathy lesions plays an important role in the narrowing of pulmonary arteries via fibrocellular intimal proliferation and thrombus formation [7,63,130,131]. OPN and VEGF are elevated in pulmonary arterial hypertension [59]. VEGF levels are an indicator of disease

Table 3
Effects of anti-cancer treatments. OPN and VEGF are variably influenced by individual drugs, combination chemotherapy, and experimental gene therapy approaches.

Treatment	Cancer	OPN	VEGF	Effect	Reference
Individual drugs					
Lang-du roots	Melanoma	Suppress	Suppress	Tumor suppression	144
Curcumin	Breast cancer	Suppress	Secondary suppress	Inhibition of motility and angiogenesis	112
Andrographolide	Breast cancer	Suppress	Suppress	Apoptosis	65
Berbamine	Breast cancer	Secondary suppress	Secondary suppress	Suppresses growth, angiogenesis, invasion	116
Topiramate	Lung cancer	Suppress	Suppress	Anti-tumor and anti-metastatic effects	108
PARP-1 inhibitors	Hepatocellular carcinoma	Suppress	Suppress	Limits tumor growth and vasculogenesis	135
Zoledronic acid	Osteosarcoma	Suppress after transient increase	Suppress	Reduces malignancy by stimulating the differentiation	146
Sunitinib		Increase	Increase	Systemic, tumor-independent multi-organ response	151
Anti-endoglin	Rapidly proliferating cells	Increase	Increase: -A, suppress: -D		152
Bispecific antibody to VEGF and OPN		Suppress	Suppress	Inhibition of tumor growth and metastasis	150
Combination chemotherapy					
Tirapazamine (plus carboplatin/paclitaxel)	Non-small cell lung cancer	No benefit	No benefit	Induction of DNA damage and cell death	92
VMDT	Myeloma	Suppress	Suppress	Reduction in angiogenesis and abnormal bone remodeling	149
Gene therapy					
OPN-directed RNA aptamer	Breast cancer	Blockade of receptor binding	Secondary suppress	Anti-proliferative, anti-metastatic, pro-apoptotic effects	109
OPN knock-down	Colon cancer	Suppress	Secondary suppress	Decrease in growth and invasion	111
OPN knock-down	Breast cancer	Suppress	Secondary suppress via decrease in HIF-1 alpha	Increased radio-sensitivity	85
OPN-directed siRNA		Suppress	Secondary suppress	Inhibits tumor growth, invasion, and angiogenesis	117
OPN-directed siRNA	Gastric tumor	Suppress	Secondary suppress	Decrease in tumor growth and metastasis	110
OPN-directed siRNA	Gastric tumor	Suppress	Independent	Reduced proliferation, migration and micro-vessel density	50
Interferon-alpha1/SG600-IL-24 gene therapy		Suppress	Secondary suppress	Anti-tumor activity	136

severity in idiopathic pulmonary fibrosis. While OPN is also up-regulated, it is not sufficient to distinguish the condition from other interstitial lung diseases [153]. In diabetic retinopathy, inflammation of the retinal micro-vessels is accompanied by an increased production of VEGF and OPN, induced by the inflammatory mediators TNF-α and interleukin-1β. VEGF as well as intact and cleaved OPN are elevated in the vitreous fluids from proliferative diabetic retinopathy patients [60, 154]. By contrast, a decrease in VEGF and OPN expression levels occurs at the earlier stage of diabetes in kidney endothelial cells. The reduction in OPN and VEGF levels leads to a suppression in cell migration and capillary morphogenesis, thus setting the stage for endothelial cell dysfunction and renal vascular complications at the later stages of diabetes [155] (Table 4).

3.3.2. Induction of OPN by VEGF

In tissue remodeling, angiogenesis contains a component of inflammation. In injured arteries, signaling in adventitial fibroblasts by VEGF through its receptor VEGFR1 induces OPN expression through the ERK1/2 pathway. It stimulates macrophage chemotaxis during vascular inflammation and neo-intima formation. VEGF thus contributes to accelerating the vascular wound healing and remodeling process [22, 24]. Externally added VEGF affects OPN expression by shifting macrophage infiltration to an earlier time point after arterial injury, leading to earlier OPN secretion by macrophages, thus increasing adventitial angiogenesis and promoting repair, while reducing vascular remodeling, aortic calcification and acute hypoxia after vascular injury [23].

3.3.3. Regulation of VEGF by OPN

OPN, released by retinal glial cells in response to glial cell line-derived neurotrophic factor, is able to inhibit the hypo-osmotic cell swelling that is a pathogenic factor in retinal edema formation. OPN activates a cell-volume regulatory signaling cascade that acts via VEGF production. Upon activation, VEGF stimulates the release of glutamate, ATP and adenosine, which are responsible for the efflux of potassium and chloride ions from these cells, thus stabilizing the transmembrane osmotic gradient [13]. After subarachnoid hemorrhage in the brain, OPN mediates its protective effects on the blood/brain barrier via up-regulation of MAP kinase phosphatase 1 (MKP-1) and suppression of MAP kinase. MAP kinase acts both upstream and downstream of VEGF-A, by inducing VEGF-A expression and by mediating the effects of VEGF-A; it thereby promotes blood/brain barrier disruption. Thus, inhibition of endogenous OPN leads to a reduction in MAP kinase phosphatase 1 levels and up-regulates both MAP kinase and VEGF-A, causing an increase in blood/brain barrier permeability [53].

3.3.4. Independent actions of OPN and VEGF

OPN plays an important role in inflammation and atherogenesis, along with CD68, tissue factor, and VCAM-1. Its expression is strongly correlated with glucose uptake in the progression of atherosclerosis on a high-fat diet. By contrast, there is no correlation of VEGF, along with its upstream hypoxia markers HIF-1α and HIF-2α, to glucose uptake and atherogenesis [41].

Heart hypertrophy during pregnancy is associated with increased myocardial angiogenesis and VEGF up-regulation. Both capillary density and VEGF levels return to non-pregnant levels postpartum. However, the hypertrophic heart is neither associated with fibrosis nor with a fetal gene expression program that includes OPN expression [42].

In the treatment of peripheral artery disease with intermittent claudication, the ACE inhibitor ramipril increases the levels of angiogenic VEGF-A while decreasing the levels of inflammatory markers like OPN, thus increasing the walking ability in these patients [156].

3.4. Immune and inflammatory conditions

OPN and VEGF are important for vascularization in inflammatory diseases, and both are elevated in arthritis and rheumatism. At the site of joint inflammation in patients with juvenile idiopathic arthritis, OPN and VEGF are induced by hypoxia. There is a positive correlation between vascular density and the expression of these cytokines in the synovial fluid [61,80]. Osteoblasts from sclerotic zones of osteoarthritic

Table 4
OPN and VEGF in vascular diseases. OPN and VEGF may synergize in angiogenesis. Their effects can be protective in some conditions, but contribute to pathophysiology in others.

Physiology
Neo-angiogenesis
Disease protection
Retinal edema
Subarachnoid hemorrhage
Pathophysiology
Thrombotic microangiopathy (narrowing of arteries)
Pulmonary arterial hypertension
Idiopathic pulmonary fibrosis
Diabetic retinopathy (opposite in diabetic nephropathy)

sub-chondral bone express significantly higher levels of VEGF and OPN than osteoblasts from non-sclerotic bone. Whereas the gene expression of VEGF is rather constant the expression of OPN increases over a period of weeks [157]. The process of valvular calcification in rheumatic heart disease is similar to bone formation and is accompanied by inflammation. This is reflected in the presence of OPN and osteocalcin within proliferating myo-fibroblasts. The release of VEGF occurs along with neo-angiogenesis [158].

Pulmonary pathologies typically entail a component of inflammation. The levels of OPN in exudative pleural effusions, due to either pleural inflammation or malignancy, are higher than those present in the blood or transudative pleural effusions. These levels correlate not only with VEGF levels in pleural fluid but also with other markers of inflammation, including pleural fluid lactate dehydrogenase [9]. OPN is augmented in idiopathic pulmonary fibrosis, induced by TGF-α, and increases during disease progression. While OPN expression persists beyond the presence of TGF-α, the levels of VEGF-A and VEGF receptor decrease with a concomitant increase in the angiostatic protein SERPIN-F1, suggesting that angiogenesis is suppressed in advanced pulmonary fibrosis [48]. In the absence of OPN, the exposure to allergen causes lesser lung fibroblast proliferation, reduced collagen deposition, diminished inflammatory cell accumulation, and decreased expression of VEGF, TGF-β and MMP-2 than is the case in OPN competent hosts [17]. Aerobic training in asthma reduces the levels of inflammatory mediators and proteins associated with the induction of airway remodeling, including OPN and VEGF, thus preserving airway integrity [159]. Within hours of infection with *Mycobacterium tuberculosis*, macrophages express genes involved in cell homing, including interleukin-8 and OPN. VEGF and its receptor VEGFR2 are also up-regulated and match the migration of the cells to the site of infection [160].

Inflammation is a pathogenetic component of eye injury as well as diabetic retinopathy. In retinal endothelial cells, exposure to the inflammatory cytokines TNF-α and IL-1β leads to elevated VEGF and OPN expression. However, VEGFR-1 is up-regulated only in response to TNF-α, but not IL-1β. The STAT3 pathway is activated. It participates in angiogenesis in part through the modulation of VEGF expression. Elevated levels of extracellular matrix proteins, like OPN, collagen IV and tenascin-C, is concomitant with activation of the MAP Kinase and NF-κB pathways. VEGF mediates ocular inflammation, neovascularization and vascular permeability, while extracellular matrix proteins are involved in tissue remodeling, cell migration and vascular inflammation [60]. In ocular fibroblasts, OPN enhances TGF-β1 mediated signaling via SMAD3 and P38 and increases VEGF expression, leading to corneal neovascularization in response to injury [68].

VEGF and OPN are secreted by receptive endometrium, macrophages and dendritic cells. In response to an inflammatory response due to local injury in endometrium, OPN levels increase without any change in VEGF expression, thus aiding in the process of implantation and angiogenesis [161].

Auto-antibodies against VEGF and OPN are differentially expressed. Whereas anti-cytokine auto-antibodies against VEGF are ubiquitously present, auto-antibodies against OPN are variably produced by healthy individuals [162].

Conflict of interest

The authors declare no conflict of interest.

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