Osteopontin as a biomarker for osteosarcoma therapy and prognosis (Review)

XINGWEN HAN[1], WENJI WANG[1], JINGJING HE[2], LEI JIANG[3] and XUN LI[4]

[1]Department of Orthopedics, First Hospital of Lanzhou University; [2]Department of Liver Diseases, Second Hospital of Lanzhou University; Departments of [3]Oncology Surgery and [4]General Surgery, First Hospital of Lanzhou University, Lanzhou, Gansu 730000, P.R. China

Abstract. Osteosarcoma (OS) is the most common bone malignancy, and is particularly prevalent in children and adolescents. OS is an aggressive tumor with a tendency to metastasize and invade to para-carcinoma tissues. The primary treatment for this tumor is a combination of surgery and chemotherapy. However, the prognosis remains poor due to chemoresistance and early metastasis. Osteopontin (OPN), a multifunctional secreted protein, has emerged as an important potential biomarker for diagnosing and treating cancer. The overexpression of OPN has been found in numerous malignant tumors, including breast, lung, gastric and ovarian cancer, as well as melanoma. Recent studies have suggested that OPN may provide an important function in the diagnosis and treatment of OS. The present review summarizes current knowledge and progress in understanding the potential role of OPN as a biomarker in OS.

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Correspondence to: Professor Xun Li, Department of General Surgery, First Hospital of Lanzhou University, 1 Donggang West Road, Lanzhou, Gansu 730000, P.R. China
E-mail: lxdr21@126.com

1. Introduction

Osteosarcoma (OS) is the most frequently occurring bone malignancy and the second leading cause of cancer-associated mortality in children and adolescents (1). The worldwide OS incidence rates are 4 and 5 cases per million individuals per year at the ages of 0-14 and 0-19 years, respectively. The incidence rate is higher in males than females (5.4 vs. 4.0 cases per million individuals per year, respectively). There are two peaks in OS incidence against age, with the first peak occurring between the ages of 10 and 14, and coinciding with the rapid development period of adolescence, indicating a strong association between adolescent growth and OS. The second peak occurs over the age of 65 years (2). The majority of OS originates from the long bones and 50% of cases occur in the region of the knee, including the distal femur and proximal tibia (3). OS is highly invasive and has a metastatic rate of ~20%, with the most common target for metastasis being the lungs (4).

The primary treatment is a combination of surgery and chemotherapy, including removing primary tumors and occasionally distant metastatic tumors with or without adjuvant chemotherapy (5). Surgical procedures for OS patients include amputation of the limb or limb salvage, which is determined based on the stage of OS. Limb salvage is performed on patients with lower grade OS, as the prognosis is similar to that of amputation (6). The drugs used for standard adjuvant chemotherapy are methotrexate, doxorubicin and cisplatin (7-9). However, early metastasis can lead to treatment failure and mortality (10,11). The prognosis for patients with metastatic tumors is substantially poorer than that for patients with primary tumors only. The 5-year survival rate is reported to be 27.4% for patients with metastases at the initial diagnosis and 70% for patients without metastases (3).

Although the 5-year survival rate of a number of other cancer types has increased with an earlier diagnosis and improved treatments, the clinical outcomes for OS have not shown comparable improvement (12). Therefore, improvements in OS diagnosis and treatment are urgently required. The identification of a biomarker to predict early metastasis would represent a revolutionary breakthrough for OS diagnosis and treatment (13-15). Biomarkers are usually detectable in the blood or other bodily fluids, and in the tissues, and are

typically tumor type-specific or sensitive to a particular bodily response that is associated with the presence of a cancer (16-19), including α-fetoprotein in hepatocellular carcinoma, cancer antigen (CA)153 in breast cancer and CA125 in ovarian cancer diagnoses. Osteopontin (OPN) was first described as a marker of transformation of epithelial cells in 1979 (20). During the following 38 years, the role of OPN in the development of human tumors, as an indicator of malignancy and as a potential prognostic factor for clinical outcomes, has been investigated. The present review will comprehensively summarize progress in this area and propose future study directions regarding the role of OPN as a biomarker for OS based on its structure and function, as well as its association with the carcinoma.

2. Structural and functional characteristics of OPN

OPN is a chemokine-like, calcified extracellular matrix-associated protein that was first identified in bone. The multifaceted roles of OPN were intensively investigated following its discovery (21,22). Human OPN, which consists of 314 amino acid residues, is a highly negatively charged protein that appears to lack complexity in its secondary structure (23). Human OPN contains a number of highly conserved structural elements, including serine-valine-valine-tyrosine-glycine-leucine-arginine and arginine-glycine-aspartate domains for integrin binding, a calcium binding site and heparin binding domains for mediating extracellular matrix receptor III (CD44 antigen) binding (24). There are five isoforms of OPN, which are encoded by five transcript variants derived from alternative splicing of the transcript encoded by the secreted phosphoprotein 1 gene (also known as OPN). OPN-a is the full-length isoform, OPN-b lacks exon 5 and OPN-c lacks exon 4, whereas isoforms 4 and 5 lack two alternate in-frame exons. OPN is a secreted extracellular glycophosphoprotein; it is usually extensively post-translationally modified by glycosylation, phosphorylation and sulfation, plus a number of cross-linking and proteolytic processes (25-27). High expression of OPN is found in osteoblasts, osteoclasts, vascular, smooth and skeletal muscle cells, lymphocytes, endothelial cells, neural cells and certain carcinoma cells.

3. OPN expression in common solid tumors

Tumor progression is dependent on the proliferation and metastasis of tumor cells, and leads to an increased risk of mortality in patients with OS. Therefore, it is imperative that a reliable biomarker for early tumor diagnosis and treatment is found. A large number of studies on different tumor types have shown that OPN serves a unique role in the proliferation and metastasis of malignant tumor cells (Table I), indicating that OPN may be a potent biomarker for cancer. Overexpression of OPN is associated with patient survival and the effect of therapeutic treatment, including surgery, chemotherapy or radiotherapy, in lung cancer (28-37). Higher OPN levels are associated with a poor prognosis, and OPN is a predictor of malignancy and poor outcomes following neoadjuvant chemotherapy in breast cancer (38-43). An elevated OPN level is associated with lymph node metastasis, Tumor-Node-Metastasis stage, depth of invasion, tumor size and distant metastasis in gastrointestinal cancer (44-62). OPN can be used as a marker of malignancy and multidrug resistance in genitourinary tumors (63-75).

4. Signaling pathways that may be activated by OPN in common tumors

As aforementioned, OPN is overexpressed in numerous tumor types and is associated with a poor prognosis, metastasis and therapy failure, suggesting that OPN may have marked clinical value in the treatment of malignant tumors. A number of studies (76-80) have addressed the mechanisms and possible signaling pathways involved in OPN-mediated tumor malignancy. Interactions between OPN and integrin promote tumor cell growth and angiogenesis. The interaction between OPN and hypoxia inducible factor 2α (HIF2α) promotes the expression of E-cadherin and vimentin to activate the epithelial-mesenchymal transformation (EMT) pathway, which stimulates tumor cell metastasis and metastatic colonization (76). OPN regulates HIF1α-dependent vascular endothelial growth factor (VEGF) expression via integrin-linked kinase/protein kinase B-mediated activation of the p65 subunit of nuclear factor-κB (NF-κB), and thus increases tumor angiogenesis. OPN induces cytochrome oxidase subunit 2 and prostaglandin E2 secretion through extracellular signal-regulated kinase and p38 mitogen-activated protein kinase-dependent activator protein 1 activation via integrin α9β1, and thus enhances tumor cell motility and angiogenesis. OPN binds to its receptor integrin α4β1 and induces tumor relapse via the phosphorylation of inhibitor of NF-κB kinase (IKKβ), which is followed by increased nuclear translocation of p50 and p65 subunits of NF-κB (77-79). Certain studies have demonstrated that OPN stimulates cancer stem cell-mediated tumor progression by inducing high expression of CD44 isoforms containing exon v6 (CD44v6) through the WNT/β-catenin pathway (80). Fig. 1 outlines the signaling pathways by which OPN may affect tumor cell proliferation, invasion, metastasis and angiogenesis.

5. OPN expression in OS

Expression of OPN in bone tissues is critical for the status of osteoblasts. OPN is necessary for modulating osteoblast differentiation through integrin αvβ3-mediated cell signaling (81). Reducing OPN expression inhibits the differentiation of mesenchymal stem cells or immature osteoblasts into mature osteoblasts while preserving the characteristics of immature osteoblastic-like cells, which may lead to OS (11). Changes in OPN levels may be associated with differentiation, growth and differentiation abnormalities in OS cells. A decreased level of OPN in osteoblasts is involved in the progression of OS via OPN-downregulated osteoblastic differentiation from mesenchymal stem cells (82). Lower levels of OPN expression in OS cells indicate that the majority of OS cells fail to undergo terminal osteogenic differentiation, thereby promoting OS growth (83). However, an elevated level of OPN in tumor cells or stromal cells has been reported to enhance the metastatic ability of OS (84).

The effect of OPN on the proliferation and migration of OS cells has been investigated *in vitro*. OPN overexpression stimulates OS cell proliferation in a dose-dependent manner, facilitates cyclin A expression in OS cells to accelerate the cell

Table I. Expression and role of osteopontin in common solid tumors.

Human tumor	Expression and role	Samples	Examination methods	(Refs.)
Lung cancer	Increased OPN was associated with patient survival and the effect of treatment	Tumor tissue, patient plasma and normal tissues	RT-qPCR and/or western blot analysis	(28-37)
Breast cancer	Increased OPN was associated with poor prognosis. OPN served a functional role in malignancy and the prediction of outcomes following neoadjuvant chemotherapy	Carcinoma, patient plasma and control group	Western blot analysis and/or immunohistochemistry	(38-43)
Gastrointestinal cancer	Increased OPN was associated with lymph node metastasis, TNM stage, depth of invasion, tumor size and distant metastasis	Tumor tissue, normal tissue	RT-qPCR and/or western blot analysis	(44-62)
Genitourinary tumor	OPN can be used as a marker of malignancy and multidrug resistance	Tumor tissue, cell line and patient plasma	RT-qPCR and/or western blot analysis	(63-75)

OPN osteopontin; TNM, Tumor-Node-Metastasis; RT-qPCR, reverse transcription-quantitative polymerase chain reaction.
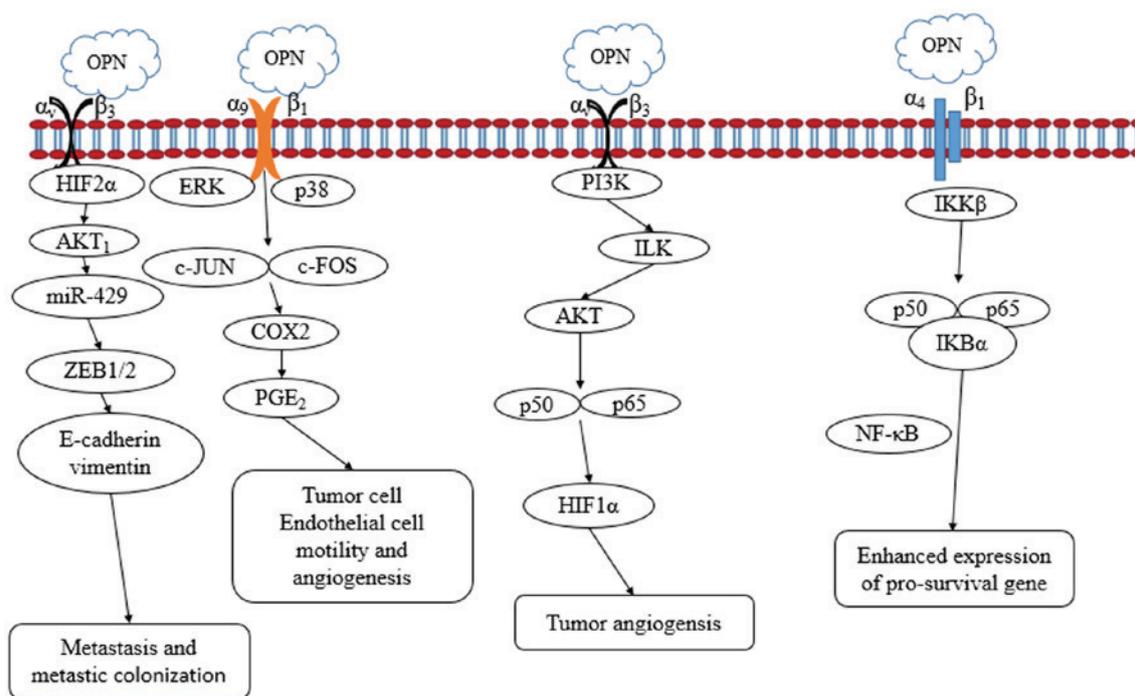
Figure 1. OPN-integrin interaction promotes tumor growth, angiogenesis and metastasis. OPN regulates HIF2α, increases the expression of E-cadherin and vimentin, and activates the epithelial-mesenchymal transformation pathway, which can stimulate tumor cell metastasis and metastatic colonization. OPN regulates HIF1α-dependent VEGF expression via inducing ILK/AKT1-mediated NF-κB p65 activation, and thus increasing tumor angiogenesis; OPN induces COX2 and PGE2 secretion through ERK- and p38-dependent c-JUN activation via α9β1-integrin, hence enhancing tumor cell motility and angiogenesis; OPN binds to its receptor α4β1 integrin, inducing the phosphorylation of IKKβ and increasing nuclear translocation of p50 and p65 subunits of NF-κB. OPN, osteopontin; HIF1α/2α, hypoxia inducible factor 1α/2α; MET pathway, MET proto-oncogene; VEGF, vascular endothelial growth factor; ILK, integrin-linked kinase; AKT1, protein kinase B; NF-κB, nuclear factor-κB; p65, NF-κB p65 subunit; COX2, cytochrome oxidase 2; PGE2, prostaglandin E2; ERK, extracellular signal-regulated kinase; p38, mitogen-activated protein kinase; c-JUN, transcription factor activator protein 1; IKKβ, inhibitor of NF-κB subunit β; p50, NF-κB DNA binding subunit; miR-429, microRNA-429; ZEB1/2, zinc finger E-box-binding homeobox 1/2; c-FOS, FOS proto-oncogene; PI3K, phosphoinositide-3-kinase; IKBα, inhibitor of NF-κB subunit α.

cycle and prompts transmembrane migration of OS cells (85). OPN also promotes the formation of OS *in vivo*. Overexpression of OPN antisense RNA in OS-732 cell xenografts was found to reduce the tumorigenicity of OS-732 cells in nude mice (86).

The small calcium-binding protein S100A4 is associated with tumor metastasis progression. Extracellular S100A4 may increase expression of the enzymes of the plasminogen activator system and matrix metalloproteinase (MMP) family,
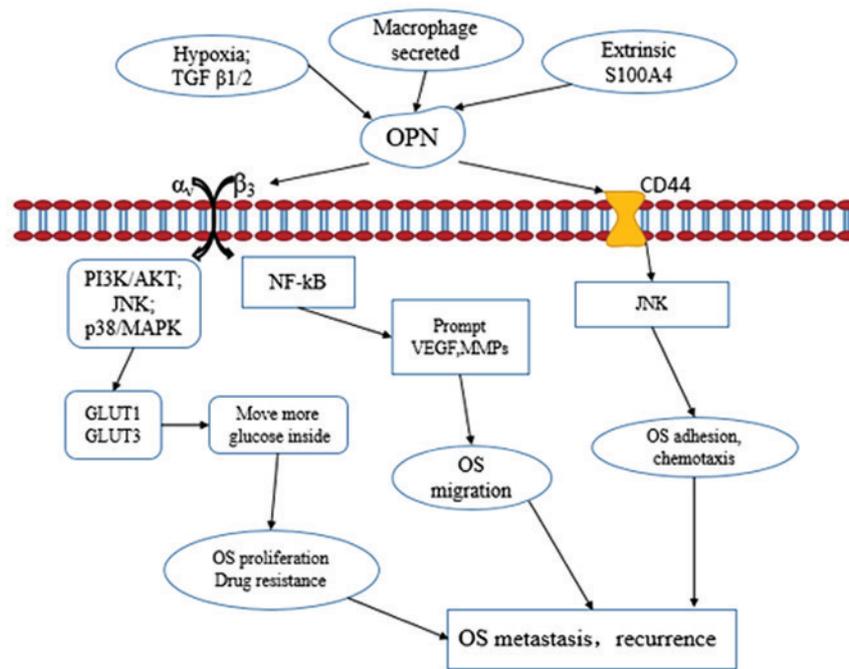
Figure 2. Central role of OPN in enhancing cell proliferation, drug resistance, migration and adhesion in OS. Various factors, including hypoxia, TGFβ1/2 and S100A4 increase OPN level to augment the expression of GLUT1/3, VEGF and MMPs through integrin and/or CD44 pathways, enhancing proliferation, drug resistance, migration and adhesion in OS, and thus contributing to OS metastasis and/or recurrence. OPN, osteopontin; OS, osteosarcoma; TGFβ1/2, transforming growth factor β1/2; S100A4, calcium-binding protein A4; GLUT1/3, glucose transporter 1/3; VEGF, vascular endothelial growth factor; MMP, matrix metalloproteinase; CD44, extracellular matrix receptor III; PI3K, phosphoinositide-3-kinase; AKT, protein kinase B; JNK, c-JUN N-terminal kinase; p38, mitogen-activated protein kinase; NF-κB, nuclear factor-κB.

particularly urokinase plasminogen activator and MMP-13. S100A4 increases the mobility and invasion of OS cells *in vitro*. S100A4 siRNA molecules inhibit OPN expression and reduce protease expression and invasion capacity in OS cells, suggesting that OPN is a downstream target of S100A4 signaling, and that OPN may also be associated with OS metastasis (87). Hypoxia is a major regulator of tumor development and aggression (88). Glucose is a source of metabolic energy that maintains the proliferation and survival of tumor cells. Glucose transporters (GLUTs) move glucose into the cytoplasm to promote aerobic glycolysis, also known as the Warburg effect (89,90). A hypoxia-mimetic agent was found to promote the expression of OPN, GLUT1, GLUT2 and GLUT3. Exogenous OPN may stimulate expression of GLUT1 and GLUT3, increasing glucose uptake into hypoxic OS cells and enhancing OS cell viability (91).

MicroRNA-4262 (miR-4262) has been identified as a key regulator of tumorigenesis, cancer cell growth and metastasis in OS. The expression of miR-4262 in OS tissue samples is decreased and the level of OPN is increased compared with matched adjacent non-tumor tissues. In addition, miR-4262 and OPN are negatively correlated in OS specimens. Overexpression of miR-4262 was found to inhibit OPN-mediated cell invasion, whereas miR-4262 depletion increased OPN-mediated cell invasion in OS cells (92). As aforementioned, studies have shown that OPN is abnormally expressed in OS, and it is associated with the proliferation, metastasis and prognosis of the disease. OPN may be used as a biomarker of the prognosis and metastasis of OS. However, identifying the specific mechanism of its action requires further investigation. These observations indicate that the altered expression of OPN may be associated with OS progression and metastasis. Fig. 2 outlines the possible signaling pathways through which OPN may affect OS metastasis and recurrence.

6. OPN as a biomarker in OS prognosis and therapy

OS is a highly malignant tumor, and the majority of patients undergo metastasis prior to diagnosis, resulting in a poor prognosis (12). OPN serves a role in metastasis and prognosis in several malignant tumors. However, our current understanding regarding the use of OPN as a biomarker for OS is insufficient. Transforming growth factor-β1 (TGF-β1) regulates several extracellular matrix proteins and promotes the expression of OPN, increasing the malignancy of OS cells (93). In a study with 11 OS patients and 29 healthy controls, mRNA levels of osteocalcin, osteonectin, OPN and type I collagen in peripheral blood samples were increased in 91% of OS patients, but were increased in only 35% of healthy subjects. Additionally, 6 OS patients with peripheral blood OPN mRNA expression exceeding the highest level found in healthy subjects developed clinical metastasis within 12 months after diagnosis. Elevated peripheral blood OPN mRNA level may result from an increased number of circulating OS cells. These observations indicate that peripheral blood OPN level may be used as a biomarker for diagnosing OS micrometastases and evaluating prognosis (94). By contrast, another study found that OPN expression in bone biopsies could not provide predictive information regarding outcomes in OS patients. Bone specimens from 57 OS patients and 11 osteoblastoma patients were used to analyze the expression of OPN and VEGF with immunohistochemistry. In OS samples, OPN and VEGF expression

were correlated with each other. High VEGF expression in OS patients showed a tendency to shorten overall survival time, but OPN had no influence on patients overall or disease-free survival times (95). The discrepancy between the two studies may be due to differences between OPN mRNA versus protein expression, the type of tissue in which OPN was measured and the evaluation of clinical outcome parameters, including metastasis or survival period. Peripheral blood OPN has the potential to be useful as a biomarker for OS and should be further evaluated in well-controlled studies.

7. Future directions

Although the expression level of OPN in OS biopsies does not appear to be a prognostic marker for OS (95), peripheral blood OPN expression has the potential to be a useful biomarker for OS (94). However, substantial research is required to validate the role of peripheral blood OPN expression level as a biomarker for OS. A reliable method to detect the expression level of OPN in peripheral blood is required. A clinical study using sufficient blood samples from OS patients and healthy controls should be conducted, and a standard reference value of OPN in the blood should be obtained through analyzing the expression of OPN in normal blood samples. The association between OPN and the prognosis of the patients with OS must also be validated. Such a study could provide answers to the following issues: i) Whether elevated OPN in the peripheral blood is an outcome of increased circulating OS cells; and ii) whether elevated OPN in the peripheral blood is correlated with the number of circulating OS cells, EMT status, metastasis, OS grade, disease-free survival rate or any other clinical parameters.

OPN is a secreted protein that may be derived from the primary OS tumor, but the presence of RNase makes OPN mRNA unstable in the blood. Therefore, methods for assessing OPN protein, such as ELISA, should be evaluated to detect OPN in patient blood specimens. Validation of peripheral blood OPN expression as a predictive prognostic marker for OS may improve clinical outcomes and quality of life for patients with OS.

8. Conclusion

The high degree of malignancy and early metastasis underscore the urgency of finding a sensitive marker to improve the diagnosis, treatment and prognosis of patients with OS. The preset review focuses on the potential value of OPN in peripheral blood as a biomarker for OS. OPN may be used as a biomarker for early diagnosis, therapeutic effectiveness and prognosis in a number of other tumors. OPN serves an important role in OS cell proliferation, invasion and migration *in vitro*, and in mice xenografts. In clinical studies, peripheral blood OPN has also been associated with micrometastases in patients with OS. However, the role of peripheral blood OPN in diagnosis, therapeutic evaluation and as a prognostic biomarker for OS must be further validated in well-controlled clinical studies.

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Availability of data and materials

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Authors' contributions

JH performed the data collection. WW and XL conceived the study, analyzed the data, and drafted the manuscript. LJ critically revised the manuscript. XH was responsible for the conception and writing of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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