REVIEW

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# Osteopontin b and c Splice isoforms in Leukemias and Solid Tumors: Angiogenesis Alongside Chemoresistance

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## Abstract

Osteopontin (OPN) is a glycoprotein involved in regulation of various influences on tumor progression, such as cellular proliferation, apoptosis, angiogenesis, and metastasis. Vascular endothelial growth factor (VEGF) is a secreted molecule supporting angiogenesis in various cancers through activation of the PI3K/AKT/ERK1/2 pathway. OPN and VEGF have a number of isoforms with various activities. In spite of the well-defined association between OPN and VEGF isoform expression and cure rate for solid tumors, there is a scarcity of information as to any association in leukemia. Based on the critical role of OPN in cell survival, it seems reasonable to hypothesize that OPN and VEGF isoform expression levels may impact on chemoresistance and relapse in leukemia the same as in solid tumors. Hence, the aim of our review was to explain relationships between OPN and VEGF isoforms and angiogenesis and related pathways in chemoresistance of leukemia and solid tumors. Our findings demonstrated that OPNb and OPNc alongside with VEGF isoforms and other gene pathways are involved in angiogenesis and also might promote chemoresistance and even recurrence in leukemia and solid tumors. To sum up, targeting OPN isoforms, particularly b and c, might be a novel therapeutic strategy for the treatment of leukemia as well as solid tumors.

**Keywords:** Osteopontin- splice isoforms- leukemia- solid tumors- angiogenesis

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## Introduction

*Osteopontin Structure*

Osteopontin (OPN) is a glycoprotein with 314 amino acids which is investigated in osteoblasts for the first time. OPN is also recognized as BPP, BSPI, TAP, ETA-I, TSP1, USP and SPP1 terms. It is expressed in multiple tissue types and secreted form could be involved in cell attachment and signal (Zahedpanah et al., 2016b; Mohammadi et al., 2017a).

The Human OPN gene is encoded by a single-copy gene on chromosome 4q21-q25 with seven exons (Young et al., 1990) (Figure1). It contains numerous highly conserved extracellular structural domains, including; aspartate domain, RGD sequence, SVVYGLR sequence, thrombin cleavage site, calcium-binding domain and heparin interconnecting domain (Anborgh et al., 2010; Cao et al., 2012). Likewise, protease cleavage sites are probably pivotal in adjusting its activity (Figure 2).

It is well proved that OPN assists as a substrate for thrombin and matrix metalloproteinases like MMP2, MMP3, MMP7, MMP9, and MMP12. Therefore, it can bind to the extracellular matrix proteins, fibronectin, and collagen. OPN was recognized with seven protein interactions: ITGAV, IGFBP5, PDLIM7, CD44, ITGA5, CTNNBL1, and SGTA (Ramaiah and Rittling, 2007). It binds to cell surface receptors through integrins such as vitronectin receptor (Mazzali et al., 2002) αVβ3, α4β1, α9β1, CD44 (Smith et al., 1999; Zahedpanah et al., 2016b) as well as to extracellular matrix components such as collagen and matrix metalloproteinase. Integrin receptors identified with both the RGD and the SVVYGLR motifs (Ramaiah and Rittling, 2007).

*Osteopontin in Cancer*

OPN is involved in proliferation, migration, and cellular invasion, as well as preventing cell apoptosis and autophagy with binding integrin αVβ3. It has been joined with some markers in various types of cancer that are known as functionally converging biomolecules (Weber, 2011). The excessive expression of OPN in invasive cancer cells indicates a key role of OPN in cancer progression (Thalmann et al., 1999; Furger et al., 2001). The relationship of OPN with various cancers and stages of disease progression is an important goal in interfering of OPN in the treatment process. Diverse levels of OPN are reported in some cancers containing, leukemia (Zhang et al., 2010a; Liersch et al., 2012; Mohammadi et al.,

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2017c), pancreatic (Koopmann et al., 2004; Kolb et al., 2005; Hsu et al., 2010a; Collins et al., 2012), breast cancer (Patani et al., 2008; Wang et al., 2008; Macri et al., 2009), prostate cancer (Caruso et al., 2008), renal cell carcinoma (Ramankulov et al., 2007; Matušan-Ilijaš et al., 2011), gastrointestinal stromal tumors (Hsu et al., 2010c; Hsu et al., 2010b), lung cancer (Zhang et al., 2010b; Shojaei et al., 2012) and ovarian cancer (Blasberg et al., 2010b).

OPN induces anti-apoptotic effect through up-regulation Mcl-1 in gastrointestinal stromal tumors (Hsu et al., 2014). By using the specific siRNA for knockdown of OPN, inhibits αVβ3 prompted cell metastasis and stimulated the PI3K/AKT/mTOR pathway (Zhao et al., 2011; Chang et al., 2012; Saleh et al., 2016; Huang et al., 2017). OPN stimulates apoptosis via CD44 receptors, mitochondrial death pathway, and ER stress stimulates apoptosis (Zheng et al., 2012; Dalal et al., 2014). Taking all the mentioned points in accounts, OPN has an important role in the progression of the tumor with its biological function. Therefore, the targeted reduction of OPN can decrease tumor progression and cancer. Our previous study demonstrated that parthenolide induces apoptosis in U937 as a committed progenitor AML cell line via a reduction in OPN gene (Zahedpanah et al., 2016b). In another report, we suggested that knockdown of OPN inhibited apoptosis in leukemic stem cells. Also, silencing of OPN using siRNA against OPN significantly decreased colony numbers in primary CD34+/CD38- AML cells (Mohammadi et al., 2016a) (Table 1).

*OPN Splice Variants*

Different OPN variants can be detected in various human cancers. The three splicing isoforms of OPN cDNA have been recognized in humans, but there is not much available information about their function and roles of each isoform in various cancers. OPNa (NM_001040058) demonstrates the full-length cDNA, whilst OPNb (NM_00058) contains a deletion at exon 5, and OPNc (NM_001040060) contains a deletion at exon 4(Young et al., 1990; Goparaju et al., 2010). Since exon 4 is transcribed in OPNa but is absent in OPNc, it can be considered as a goal for the specific inhibition of OPN in numerous cancers (Fig.3). The various functions of the three isoforms of OPN are presented in several studies. OPNa and OPNb were expressed meaningfully in tumor and non-tumor ovarian samples, while specifically OPNc was expressed in ovarian tumor samples.

Tilli et al., (2011) presented that OPNc isoform via activation of PI3K/Akt signaling pathway plays a key role in tumorigenesis, metastasis, and invasion in the OvCar-3 cell (ovarian cancer) and IOSE cells (ovary epithelial) . Tang et al., (2013) demonstrated that gastric cancer cell survival strongly promoted by OPNb isoform. Furthermore, OPNc efficiently stimulated gastric cancer metastatic activity via increased secretion of MMP-2, IL-8, and uPA. Goparaju et al., (2010) in a recent study on OPN splicing isoforms in lung cancer cells demonstrated that 91% of NSCLC tumors had an increased OPNa expression. Meaningfully overexpression of OPNa increased activity in scratch closure and proliferation. On the contrary, overexpression of OPNc decreased activity in proliferation and invasion assays. Based on Mirza et al., (2008) report, OPNc is a diagnostic and prognostic marker in breast cancer alongside contractual breast cancer markers. Thus, OPN splice variants differentially exert clinic-pathological features and biological functions in cancers. Consequently focused on specific OPN splicing isoform might lead to developing diagnostic and therapeutic approaches to various cancers (Table 2).

*Molecular Mechanisms of OPN in Cancers*

Various OPN function in cancer is performed via direct connection of OPN with integrin or/and CD44 which lead to activation of different pathways in malignant cells (Liaw et al., 1995; Irby et al., 2004; Bellahcène et al., 2008). OPN molecule is able to activate several signaling transduction pathways via a complex signaling network in various cancer models such as prostate cancer, gastrointestinal cancers, melanoma, myeloma, breast cancer and lung cancer (Bellahcène et al., 2008). For instance, a recent report showed that tumor-promoting functions of OPN are related to NF-kB/ PI3-K/Akt pathway activation with subsequent up-regulation of BCL-XL in solid tumors (Lee et al., 2007; Sun et al., 2008; Zhao et al., 2008).

Our previous study in leukemia cells showed that OPN plays a role in activation the signal pathway of the PI3K/Akt, following the binding to avβ3 integrin or CD44. Furthermore, OPN causes mTOR phosphorylation in Ser-2448 (Zahedpanah et al., 2016a; Mohammadi et al., 2017d) (Figure 4). Previously in some studies, we reported that in parallel with OPN knockdown by using siRNA, AKT/mTOR/ PTEN/β-catenin expression levels were significantly decreased in AML cell lines and also enhanced apoptosis in AML blasts and CD34+/CD38-/CD123+ leukemic stem (Mohammadi et al., 2016a;

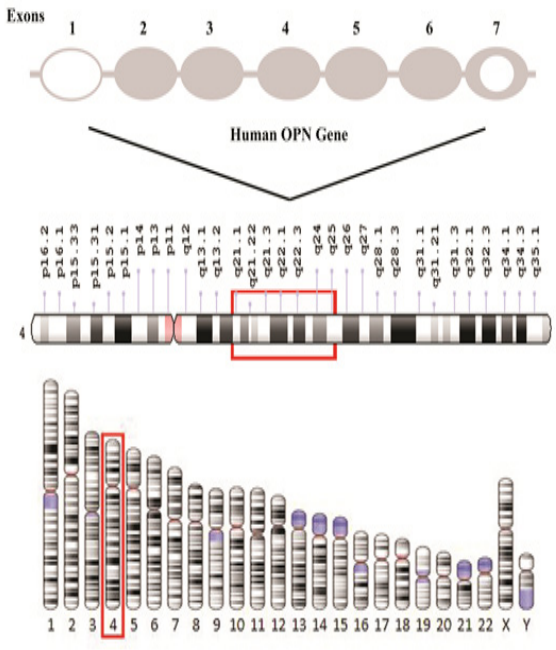


Figure 1. The Gene Structure of Human Osteopontin on Chromosome 4. Exons are shown as boxes, coding regions are filled boxes and un-translated regions are empty boxes.

Table 1. Summary of OPN Functions and Clinical Significance in Human Cancers.

| Cancer type | Biological functions | Clinical significance | Refs. |
|---|---|---|---|
| Prostate | - hOPN mRNA was over-expressed in both human prostate cancer cell lines and in clinical human specimens.<br>- Expression of OPN and IL-8, are related to metastasis and angiogenesis in prostate cancer. | - OPN is paracrine and autocrine mediator of growth and progression in prostate cancer.<br>- In prostatectomy specimens, expression of OPN is related to disease recurrence. | (Thalmann et al., 1999; Caruso et al., 2008) |
| Leukemia | - PI3K/AKT signaling by Tax protein of HTLV-1 might induce leukemogenesis.<br>- Serum OPN increased in chronic and acute hematologic malignancy.<br>- Curcumin as a NF-kB inhibitor can be prompted apoptosis and inhibited proliferation of AML cell lines and primary isolated AML cells.<br>- Parthenolide decreases protein level of OPN and mRNA expression of AKT1, mTOR, PTEN, and β-catenin in AML cells. | - The level of OPN expression is known as an independent prognostic marker in AML.<br>- Parthenolide has several applications including apoptosis and reduced expression of OPN gene in AML cells.<br>- OPN knockdown by OPN siRNA decreased colony numbers in the Leukemic stem cells. | (Furger et al., 2001; Zhang et al .,2010a; Liersch et al., 2012; Zahed panah et al., 2016; Mohammadi et al., 2016a;Mohammadi et al., 2017c) |
| Pancreatic | - OPN is a candidate tumor marker for pancreatic cancer.<br>- Overexpression of OPN may be related to aggressive phenotype. | - OPN might be a diagnostic marker in patient's serum with pancreatic cancer.<br>- Knockdown of OPN can be used as an effective therapeutic strategy. | (Koopmann et al., 2004; Kolb et al . , 2005;Collins et al., 2012) |
| Renal cell carcinoma | - OPN via P65/NF-κB signaling pathway may be involved in the clear cell renal cell carcinoma progression.<br>. | - High OPN plasma levels are related to poor prognosis and metastases in renal cell carcinoma patients. | (Ramankulov et al., 2007; Matušan-Ilijaš et al., 2011) |
| Breast | - Overexpression of OPN has been related to tumor progression in breast cancer. | - Overexpression of OPN isoforms (OPNc) was related to poor prognosis, tumor grade, and stage of triple negative breast tumors. | Patani et al., 2008; Wang et al., 2008; Macri et al., 2009; Saleh et al., 2016) |
| Gastrointestinal stromal tumors | - OPN has anti-apoptotic effects towards imatinib in gastrointestinal stromal tumors. These effects are mediated by Mcl-1 up- regulation through β-catenin pathway.<br>- Mitosis in gastrointestinal stromal tumor is related to OPN/CD44 interaction. | - Expression of OPN was related to poor prognosis in gastrointestinal stromal tumor malignancies. | (Hsu et al., 2010b; Hsu et al., 2014) |
| Non-small-cell lung | - Expression of OPN in non-small-cell lung cancer was strongly related to tumor proliferation.<br>- OPNa binding to recombinant integrin αvβ3 efficiently inhibited by AOM1. | - Overexpression of OPN in tumor samples was related to metastasis of non-small-cell lung.<br>- A level of OPN in early-stage of non–small-cell lung cancer was associated with therapeutic response and survival.<br>- Carboplatin in combination with anti-OPN monoclonal antibody was used for the inhibition of the tumor growth in mice model. | (Zhang et al., 2010b; Blasberg et al., 2010; Shojaei et al., 2012) |
| Lung | - OPN has an important role in cell proliferation and lung cancer invasiveness.<br>- Expression of beclin-1 gene led to autophagy induction, cancer cell growth inhibition and angiogenesis inhibition. | - OPN is known as a marker for invasive lung cancer.<br>- Expression of both OPN and beclin-1 genes are known targets for prevailing on radioresistance via autophagy controlling. | (Zhao et al., 2011 Chang et al., 2012) |
| Colorectal | - Over- expression of OPN in colorectal cancer leads to cell progression and inhibiting cell autophagy via activating the p38 MAPK signaling pathway. | - OPN might be a therapeutic biomarker in patients with colorectal. | (Huang et al., 2017) |
| Ampullary | - Overexpression of OPN was associated with tumor-related macrophages. | - Overexpression of osteopontin in bulky Ampullary cancer predicts recurrence of the disease in patients | (Hsu et al., 2010a) |

Mohammadi et al., 2016b; Mohammadi et al., 2017b; Panah et al., 2017).

Soutto and Servetas studies revealed that TFF1 (tumor suppressor) could inhibit β-catenin and NF-κB via negative regulation of Akt/GSK-3β (Soutto et al., 2014; Soutto et al., 2015a; Soutto et al., 2015b; Servetas et al., 2016). OPN protein can trigger MEK/ERK1/2 pathway in cancer in order to stimulate growth and metastasis in the tumor (Sun et al., 2008).

The MMP family has an essential role in tumor invasion and metastasis. OPN promoted metastasis through MMP-2, MMP-7, MMP-9 and VEGF, and uPA (Sun et al., 2008; Georges et al., 2010; Likui et al., 2011). There is a positive relationship between OPN, VEGF, and COX-2, and they are able to induce angiogenesis and metastasis in cancer. COX-2 inhibitors have antitumor activity via OPN down-regulation, with occlusion of OPN regulatory network (Tang et al., 2008; Zagani et al., 2009) (Table 3).

*OPN and VEGF*

VEGF and OPN are two markers that have recently been identified as a biomarker for prognosis in various cancers (Cui et al., 2009; Mirzaei et al., 2017b). OPN and VEGF are involved in proliferation, invasion, tumor progression and finally in angiogenesis of cancer cells (Kumar et al., 2013). There is a synergistic effect between VEGF and OPN. For example, OPN can stimulate VEGF (Chakraborty et al., 2008; Dai et al., 2009; Tilli et al., 2012; Kumar et al., 2013; Wahl et al., 2013; Raja et al., 2014) by the integrin αvβ3 in umbilical artery cells (Infanger et al., 2008).

Table 2. Summary of OPN Splice Variants Functions and Clinical Significance in Human Cancers

| Cancer type | Biological functions | Clinical significance | Refs. |
|---|---|---|---|
| Breast cancer | - OPNc is correlated with proliferation and metastasis. | - OPNc is known as a diagnostic and prognostic marker.<br>- OPNc isoforms predicts grade 2-3 in breast cancer. | (Mirza et al., 2008) |
| Non-small cell lung cancer | - Expression of OPNa was increased in most non-small cell lung cancer tumors. | - An aggressive phenotype and an indolent phenotype of Non-small cell lung cancer were produced by OPNa and OPNc respectively. | (Goparaju et al., 2010) |
| Ovarian | - Cell proliferation, invasion, migration and tumor formation are activated by OPNc in in vivo model.<br>- OPNc was encouraged immortalized ovarian epithelial cells and have an important role in ovarian cancer tumorigenesis. | - The expression of OPNc participates in ovarian cancer tumorigenesis and progression. | (Tilli et al., 2011) |
| Gastric | - Overexpression of OPNb and OPNc was related to gastric cancer cell survival by Bcl-2 family proteins.<br>- An expression of CD44v was enhanced gastric cancer metastatic activity via increasing secretion of IL-8, uPa, and MMP-2. | - OPN isoforms have diagnostic and therapeutic approaches in the gastric cancer cell. | (Tang et al., 2013) |

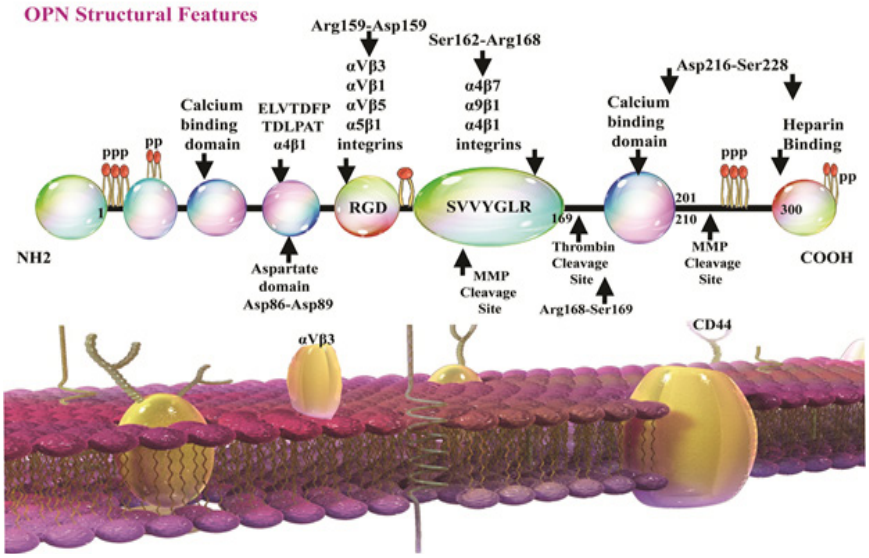


Figure 2. The Structure of Human Osteopontin Protein Demonstrates Selected Structural Domains. Structural domains is consists of (1): Aspartate domain-amino acid sequence Asp86-Asp89-binds hydroxyapatite, (2): RGD sequence amino acid sequence Arg159-Asp159-binds αVβ3, αVβ1, αVβ5 and α5β1 integrins, (3): SVVYGLR sequence amino acid sequence Ser162-Arg168-binds α9β1 and α1β1 integrins, (4):Thrombin cleavage site-amino acid sequence Arg168-Ser169-displays RGD sequence, (5): Calcium binding domain-amino acid sequence Asp216-Ser228-calcium-binding, (6): Heparin-binding domain-amino acid sequence Asp290-Ile305 - mediates CD44v3 binding.

Chakraborty et al., (2008) confirmed that OPN stimulates tumor progression and angiogenesis by activation of some pathways such neuropilin-1/Brk/NF-κB/ATF-4 signaling in various grades of cancer. Moreover, they indicated that OPN via using some mechanisms stimulates VEGF-dependent cancer progression and angiogenesis.

VEGF can stimulate integrin αvβ3, OPN, and thrombin which leads to accelerated migration activity through endothelial cells at some point of angiogenesis (Senger et al., 1996). In endothelial cells, OPN stimulates angiogenesis and induces VEGF via three main pathways including, phosphorylation and activation of PI3K/AKT and ERK1/2 cascades and mutually, VEGF induces OPN with integrin αvβ3 and thrombin (Dai et al., 2009; Ramchandani and Weber, 2015).

Our previous studies in leukemia stated that Sorafenib and Thalidomide as VEGF inhibitors in combination with arsenic trioxide and curcumin have synergistic impacts on inhibition of cell proliferation and promotion of apoptosis in AML cells without FLT3 mutation (Haghi et al., 2017; Salemi et al., 2017) (Table.4).

## Discussion

OPN splicing isoforms have been observed and measured in various cancers, including esophagus, stomach, liver, pancreas, brain, breast, head, neck, soft tissue sarcoma and AML (Lazar et al., 2010; Mirzaei et al., 2017a; Mirzaei et al., 2017b). With this regard, it has not yet been determined a clear and precise relationship between different splicing isoforms of OPN with VEGF in various cancers. As shown in previous studies, OPN splicing isoforms have different interactions with VEGF and angiogenesis in various cancers. Li et al., (2014) concluded that among all OPN splicing isoforms, OPNc

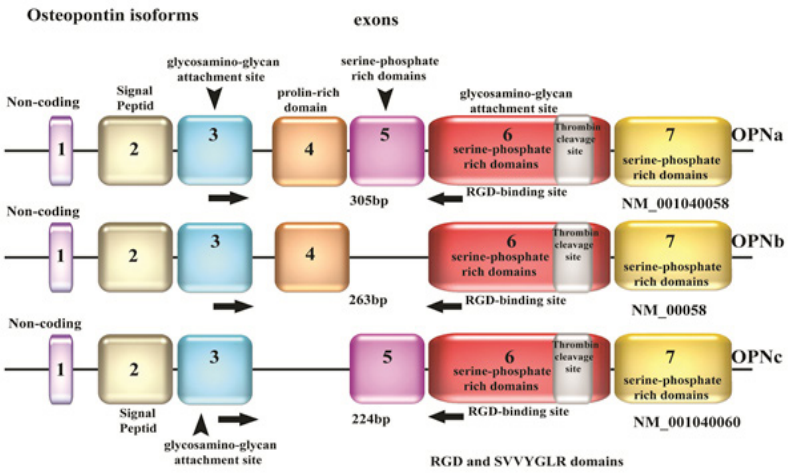


Figure 3. Different OPN Variants and Gen-Bank Accession Numbers. OPNa has six translated exons, while OPNb has a deletion of exon 5, and OPNc has a deletion of exon 4.

and OPNb, play tumorigenic roles in prostate cancer cells and also these two isoforms impel secretion higher levels of VEGF, MMP2, and MMP9 in prostate cancer cells (Tilli et al., 2012) while OPNa did not affect prostate cancer cells.

Evidence of our previous study for the first time declared that the two main OPN isoforms including OPNb and OPNc have an important role in angiogenesis and chemoresistance in AML through STAT3/VEGFC/β-catenin genes pathways. With inhibition of OPN splicing isoforms with simvastatin and special siRNA, we demonstrated a decrease in STAT3, VEGFC, and β-catenin genes that they probably are located in downstream of OPNb and OPNc isoforms (Mirzaei et al., 2017a; Mirzaei et al., 2017b). OPNa still has no role in angiogenesis in AML cell lines according to our reports and also study conducted by Li et al., (2014).

Some studies explained that OPNc decreases VEGF expression and so reduces tubule formation in non-small cell lung cancer while OPNb acts through its effect on αvβ3 receptor integrin. OPNa with αVβ3 receptor binding integrin caused VEGF secretion and increased angiogenesis (Donati et al., 2005; Goparaju et al., 2010; Zhang et al., 2010b; Shojaei et al., 2012).

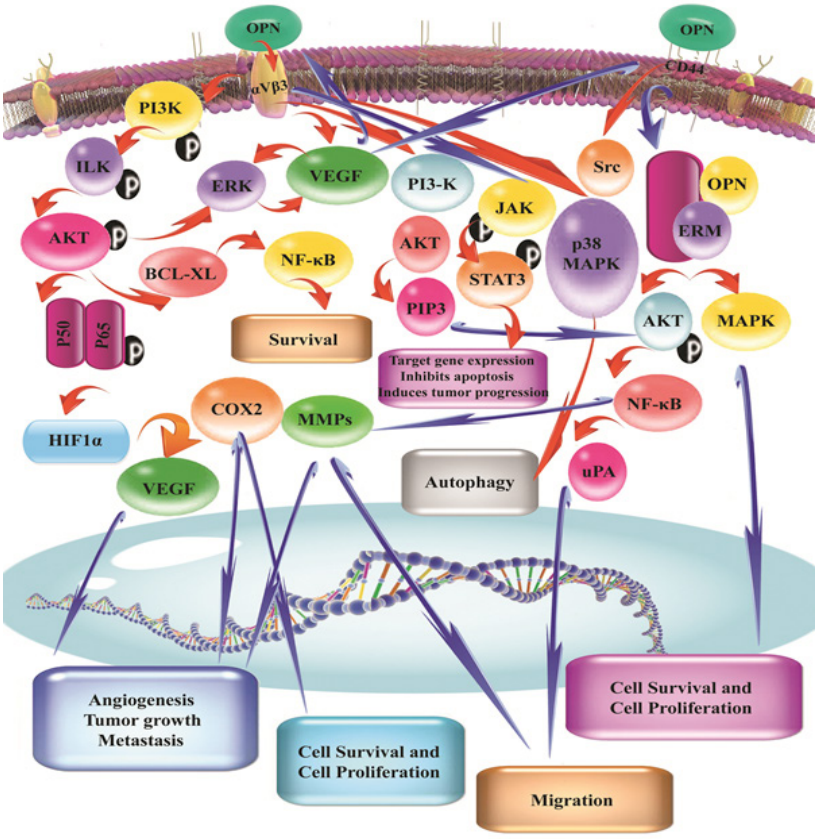


Figure 4. Molecular Mechanisms of Osteopontin. OPN in connecting with CD44 or/and integrin αVβ3 cause growth and metastasis via activation of some pathways such as cell survival, cell proliferation, angiogenesis and migration.

Table 3. Summary of Molecular Mechanisms of OPN Functions and Clinical Significance in Human Cancers

| Cancer type | Biological functions | Clinical significance | Refs. |
|---|---|---|---|
| Colon | - Overexpression of OPN via interaction with CD44 was associated with tumor stage, reduced intercellular adhesion, proliferation and increased CD31 positive microvessel counts in the colon cancer. | - OPN has critical role in human colon cancer progression. | (Irby et al., 2004) |
| Gastric | - OPN, COX-2, and VEGF Levels are associated with lymph node metastasis, stage of cancer and microvessel density in gastric cancer.<br>- β-catenin and c-Myc was detected in hyperplastic tissue and maintained all stages of gastric cancer tumorigenesis.<br>- The β-catenin/T-cell factor transcription activity in MKN28 gastric cancer cells was inhibited by reconstitution of TFF1. | - The interaction between OPN and CD44V promotes ECM-derived survival that affects progression of gastric cancer.<br>- Metastasis and angiogenesis in gastric cancer w promoted by COX-2, OPN, and VEGF. Thus they can be the goal strategy for tumor prevention and therapy.<br>- Lack of TFF1 can be a significant step in promoting the oncogenic activation of β-catenin and gastric tumorigenesis mediated by H. pylori. | (Lee et al., 2007 ; Tang et al., 2008) Soutto et al., 2014; Soutto et al., 2015a; Soutto et al., 2015b; Servetas et al., 2016) |
| Hepatocellular | - OPN can induce metastasis and tumorigenesis via inhibition apoptosis in hepatocellular carcinoma cell.<br>- Knockdown of OPN leads to down-regulation of α4, β1, and β3 integrin genes, Locked NF-kB activity, inhibits expressions of XIAP and Bcl-2 and Bcl-XL over-expression. | - Silencing of OPN by siRNA can prevent hepatocellular carcinoma cell growth and sensitized<br>these cells to chemotherapeutic agents. | (Sun et al., 2008; Zhao et al., 2008) |
| Colorectal | - Cyclooxygenase-2 inhibitor with antitumor activity was down-regulated OPN via blockade of NR4A2 and Wnt signaling pathway.<br>- Expression of OPN protein in colorectal cancer tissues was more than in non-tumor tissues. | - Parecoxib quickly down-regulate OPN in intestinal polyps.<br>- Rofecoxib was reduced the orphan nuclear receptor NR4A2 levels in adenomas from patients.<br>- Targeting OPN with RNAi can increase radio-sensitivity and suppress invasion of colorectal cancer cells | (Zagani et al., 2009; Georges et al., 2010; Likui et al., 2011) |
| Leukemia | - Upregulation of OPN, significantly up-regulate AKT/ mTOR/ PTEN/ β-catenin/ NF-κB1 expression levels in the enriched CD34+ AML cells.<br>- Osteoblasts promote enrichment of leukemic stem cell in response to daunorubicin and curcumin via OPN/ STAT-3/VCAM-1/IL-6/CXCL-12 up-regulation in AML cell lines. | - Daunorubicin in combination with curcumin has more growth inhibition effect on CD34+/ CD38- AML cells.<br>- OPN seems to be main molecular marker in AML blast and leukemic niche for targeted treatment<br>- Knockdown of OPN with siRNA increased the cytotoxic effects of parthenolide on AML cell lines and decrease AKT, mTOR, β-catenin, and PTEN expression. | (Mohammadi et al., 2016b; Mohammadi et al., 2017a; Mohammadi et al., 2017b; Panah et al., 2017) |

Table 4. Summary of OPN and VEGF Functions and Clinical Significance in Human Cancers

| Cancer type | Biological functions | Clinical significance | Refs. |
|---|---|---|---|
| Breast | - Expressions of VEGF and OPN were associated with breast tumor kinase, neuropilin-1, NF-kB, and activating transcription factor-4 signaling cascades in various stages of breast cancer.<br>- OPN induces NF-κB p65 activation that leads to expression of VEGF and HIF1α in response to hypoxia.<br>- Overexpression of HIF1α and OPN exert in pre-neoplastic and early stage of breast cancer. | - Expression of VEGF by tumor cells and neovascularization by VEGF are two important essential roles of tumor progression of OPN.<br>- OPN via expression of VEGF/ HIF1α dependent pathway and also activation of NF-κB / ILK in response to hypoxia can control angiogenesis and eventually breast cancer progression. | (Chakraborty et al., 2008; Raja et al., 2014) |
| Lung | - Knockdown of OPN can reduce the formation of malignant pleural effusion. | - OPN was involved in formation of malignant pleural effusion of lung cancer likely by promulgating VEGF secretion. | (Cui et al., 2009) |
| Endothelial | - OPN enhances the expression of VEGF via the AKT and ERK phosphorylation.<br>- VEGF induced by OPN activates PI3K/AKT and the ERK1/2 pathway. | - Anti-OPN antibody has more anti-angiogenesis effect in comparison to anti-VEGF antibody in vivo. | (Dai et al., 2009) |
| Prostate | - Overexpression of OPNc and OPNb in line with MMP-2, MMP-9, and VEGF gene expression could be enhanced invasion, migration, proliferation and tumor growth via PI3K signaling. | - Down-regulating of OPNc and OPNb expression could prevent progression of prostate cancer. | (Tilli et al., 2012) |
| Retinal Glial | - The neuro-protective effects of OPN with autocrine effects interceded by the release of VEGF from Müller cells. | - Pharmacological blockers of VEGFR2, neutralizing anti-VEGF antibody can abrogate OPN effect. | (Wahl et al., 2013) |
| Melanoma | - Expression and activation of VEGF, ABCG2, and ERK1/2 were induced by OPN.<br>- OPN derived from stroma regulates resistant phenotype via ERK2 activation in melanoma cells. | - Inhibition of OPN might be a novel therapeutic strategy for treatment of malignant melanoma. | (Kumar et al., 2013) |

Lazar et al., (2010) found that OPNc is expressed mainly in pancreatic cancer tissues and promoted by nicotine and plays its role by up-regulating the expression of MMP-9 and VEGF. Therefore, high levels of OPNc, VEGF, and MMP-9 are related to an aggressive phenotype. OPNa, VEGF, and thrombin through its effect on αvβ3 receptor integrin, and increases the secretions of VEGF by tumor cells cause progression in lung cancer (Senger et al., 1996; Blasberg et al., 2010a; Ramchandani and Weber, 2015).

It's believed that STAT3 is activated in a wide range of cancer cells (Niu et al., 2002) and can regulate metastasis in human cancer cells by promoting tumor angiogenesis (Wang et al., 2011) through the modulation of VEGF expression (Ramchandani and Weber, 2015). Wang et al., (2013) indicated that activation levels of STAT3 and IL-6 were correlated with tumor progression in neoplastic human gastric cancer cells. Many studies have shown that CXCR4/CXCL12/STAT3/VEGF molecular loop over-expression is able to promote tumor cells progression, metastasis and angiogenesis via the stimulation of PI3K/Akt pathway (Liang et al., 2007; Lin et al., 2009; Wang et al., 2011). In agreement with this finding, we have also proved that VEGF/STAT3/CXCR4 overexpression in mRNA levels in AML cell lines along with OPNb and OPNc is associated with angiogenesis. We demonstrated that simvastatin as a natural OPN inhibitor, hindered AML cell proliferation and along with inhibition of the three OPN isoforms gene expression, decrease in STAT3, VEGFC, and β-catenin gene expression. Hence, there is relevance among OPN isoforms and angiogenesis- associated genes (Mirzaei et al., 2017b; Mohammadi et al., 2017a)

In conclusion, taken together, OPNb and OPNc alongside VEGF isoforms and other genes pathways involved in angiogenesis might be promoted chemoresistance and even recurrence in leukemia and solid tumors. Likewise, OPNb and OPNc have potential to consider as molecular candidates for detection of minimal residual disease and determination of remission in AML patients. Our findings declared that targeting OPN isoforms particularly b and c in combination with anti-VEGF agents might be a novel therapeutic strategy for the treatment of leukemia as well as solid tumors.

*Abbreviations*

OPN: Osteopontin; VEGF: Vascular endothelial growth factor; ERK1/2: extracellular signal-regulated kinase 1/2 pathway; PI3-K: Phosphoinositide 3-kinase; BPP: bone phosphoprotein; BSPI: bone sialoprotein I; TAP: transformation-associated phosphoprotein; TSP1: tumor-secreted phosphoprotein 1; ETA-I: early T-lymphocyte activation; USP: Urinary stone protein; SPP1: Nephropontin and Uropontin secreted phosphoprotein 1; RGD: arginine-glycine-aspartic acid; MMP: Matrix metalloproteinase; ITGAV: Integrin alpha-V; IGFBP5: Insulin-like growth factor-binding protein 5; ITGA5: integrin alpha 5; SGTA: Small glutamine-rich tetratricopeptide repeat; Mcl-1: Myeloid cell leukemia 1; ER: endoplasmic reticulum; NF-κB: nuclear factor–inducing kinase/nuclear factor-κB; MAPK: Mitogen-activated protein kinase; uPA: Urokinase-type plasminogen activator; mTOR: mechanistic target of rapamycin; TFF1: trefoil factor 1; MEK/ERK1/2: Mitogen-activated protein kinase pathway; siRNA: small interfering RNA; COX-2: Cyclooxygenase-2; ECM: Extracellular Matrix; NR4A2: Nuclear receptor subfamily 4, group A, member 2; STAT3: The signal transducers and activators of transcription 3; CXCR4: CXC chemokine receptor 4; Brk: breast tumor kinase;; ATF-4: activating transcription factor-4

*Conflict of interest*

The authors declare no conflict of interest.

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