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The multidirectional role of osteopontin in cancer

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Osteopontin (OPN) was described for the first time as a potential marker of neoplastic transformation by Senger et al. in 1979. Studies suggesting an important role of OPN in oncology, allergology, nephrology and cardiology have been published for many years. However, the largest number of articles pertains to the role of OPN in neoplastic transformation and will surely facilitate future determination of OPN levels in blood or cancer tissues with the purpose of disease diagnosing, staging, prognosing metastases and monitoring the treatment effectiveness. Numerous studies showed that high OPN expression levels accompany metastases formation; the protein was also confirmed to be involved in stimulation of cell proliferation and formation of new blood vessels, i.e. angiogenesis.

OPN was also shown to be capable of binding the CD-44 receptors, which facilitates migration and invasion of cancer cells into the blood vessels. Correlation was also demonstrated between OPN expression and the time to disease recurrence and overall survival in patients with breast cancer, gastric cancer, hepatocellular cancer, hormone-dependent prostate cancer, kidney cancer and endometrial cancer.

The exact mechanism responsible for OPN's role in neoplastic transformation remains unclear and numerous research studies are conducted in this area.

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Key words: breast cancer, osteopontin, cancer biomarker, angiogenesis, integrin α, colorectal cancer, non-small-cell lung cancer

Introduction

Osteopontin (OPN) was described for the first time by Senger et al. (1979), who indicated its role as a potential marker of epithelial cell transformation [1]. The name of OPN is associated with its function in the bone tissue [2]. There is evidence that OPN detected in transformed cells is exactly the same protein that enables osteoclast localization in the extracellular bone matrix [3].

OPN is a highly phosphorylated and glycosylated hydrophilic protein, and it is composed of approximately 300 amino acids. The specific amino acid sequence of Arg-Gly-Asp (RGD) mediates the OPN's ability to bind to cells. Moreover, OPN has elements that bind calcium and hydroxyapatite, as well as two domains suitable for heparin [4].

Many forms of OPN of various particle size have been discovered so far, and the molecular mass of OPN ranges from 44 kDa to 75 kDa. Human OPN is encoded by a single gene consisting of 7 exons, which is located on the long arm of the chromosome 4 (4q13) [5, 6]. Initially, OPN was classified as a bone sialoprotein [7], and it was also referred to as Eta-1, secreted phosphoprotein, or uropontin [8]. The cDNA sequence that encodes OPN is similar in different mammals [9]. Recently, OPN was detected in animal milk, and it turned out that it had many similarities with human lactoferrin, which might be of importance regarding its possible use in the production of infant formulas. The variability of the native OPN structure is dependent on numerous post-translational modifications, such as glycosylation

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and phosphorylation [10]. Due to the presence of the RGD sequence, OPN belongs to a class of proteins that interact with the surface of different cell types by binding to integrin receptors [11]. Another amino acid sequence of OPN enables the interaction with cells expressing CD44 [12]. OPN binds to the cellular surface, which results in cell migration and adhesion [13].

Moreover, OPN plays a role in a number of physiological and pathological processes, such as maintenance and remodeling of bone integrity in response to tension or pressure, early cell immune reactions, dystrophic calcifications, recurrent coronary artery stenosis, regulation of growth and differentiation of cancer cells, and development of metastases [14]. It has also been shown that OPN takes part in the process of new vessel formation, i.e. angiogenesis, through a paracrine promotion of prostaglandin E2 (PGE2) binding to cell receptors on endothelial cells and other proangiogenic particles. Furthermore, OPN takes part in cell adhesion, apoptosis, inflammatory processes, and wound healing [15]. OPN is secreted into bodily fluids such as blood, urine, milk, bile and semen [16].

Under physiological conditions, OPN is expressed by osteoclasts, osteoblasts, teeth, endothelial cells of the mammary glands, kidneys, pancreas, gall bladder, bronchi, salivary glands, skin, nerve cells, endothelium, T lymphocytes, NK cells, macrophages, Kupffer cells, epithelial cells of the alimentary tract, urinary epithelium, and genital epithelium [17, 18]. An increased expression of OPN has been noted in acute and chronic inflammatory states, which is accompanied by OPN expression by T lymphocytes, dendritic cells, macrophages, and NK cells [19, 20].

OPN plays an important role in both acute and chronic inflammatory states [21] — it mediates the migration of macrophages and dendritic cells into the sites of inflammation [22], induces IL-12, IFN-γ production by macrophages and at the same time inhibits IL-10 secretion by these cells [23]. It has been determined that OPN induces an early cell-mediated immune response through the stimulation of cytokine production by Th1 lymphocytes and inhibition of Th2 lymphocytes [24]. OPN is regarded as one of the pro-inflammatory cytokines [25] and its secretion is regulated by the pro-inflammatory/anti-inflammatory cytokine system [26].

Osteopontin and angiogenesis

Vascular support is an important feature of both normal and pathological tissue structures as it enables tissue development, homeostasis with the surrounding environment by providing essential structural and energetic nutrients and oxygen. The process of new vessel formation unfolds in many stages and is regulated by numerous cytokines, enzymes, and other factors that stimulate or inhibit the formation of capillaries. Angiogenesis is a complex process that involves degradation of extracellular matrix, and subsequent activation, proliferation, and migration of endothelial cells and pericytes [27, 28]. Although angiogenesis does occur under physiological conditions (menstrual cycle, pregnancy, placenta formation, wound healing, etc.), it has been implicated in multiple disease processes such as most cancers, psoriasis, rheumatoid arthritis, endometriosis, stomach ulcers, diabetic proliferative retinopathy, and ischemic heart disease [29–34].

Recent research suggests that OPN may also reveal significant angiogenic properties [35]. OPN, through its binding to $\alpha_v\beta_3$ integrin, stimulates adhesion and migration of endothelial cells [36]. It was demonstrated that endothelial cells express $\alpha_v\beta_3$ integrin after stimulation with growth factors, in inflammatory sites, in healing wounds, and in the vasculature of certain tumors [37]. Moreover, OPN, as one of the extracellular matrix proteins, is able to inhibit endothelial cell apoptosis. The binding of OPN to $\alpha_v\beta_3$ integrin results in the activation of the transcription factor NF-κB, which plays a protective role in endothelial cell survival [38]. Furthermore, OPN directly interacts with vascular endothelial growth factor (VEGF) [39]. It has been demonstrated that VEGF causes an increased expression of $\alpha_v\beta_3$ integrin on the surface of endothelial cells, and induces OPN mRNA production. Moreover, it has been shown that OPN stimulates endothelial cell migration — endothelial cells that have been incubated with VEGF migrate faster after the addition of OPN. This might result from the fact that VEGF increases the expression of $\alpha_v\beta_3$ integrin on endothelial cell surface, which leads to an enhanced interaction with OPN [40].

Shijubo et al. (1999), in an immunohistochemical study, reported that VEGF and OPN were present in pulmonary adenocarcinoma samples and the greatest intensity of angiogenesis was associated with the presence of both VEGF and OPN [41]. Moreover, in patients undergoing surgery, VEGF and OPN were negative prognostic factors — the risk of recurrence was significantly higher in patients with a confirmed expression of either VEGF or OPN. Furthermore, a simultaneous expression of VEGF and OPN was associated with an even higher risk of recurrence. These results suggest that both VEGF and OPN have proangiogenic and pro-cancerous potential as their expression is associated with an increased risk of recurrence of pulmonary adenocarcinoma [41].

Leali et al. (2003) reported that the production of OPN is associated with fibroblast growth factor (FGF) — endothelial cells with a high expression of FGF2 are characterized by an increased expression of OPN [42].

Osteopotin and invasiveness of cancers

An increased cytoplasmic expression of OPN has been reported in lingual, esophageal, gastric, colon, pancreatic, renal, and endometrial cancer. OPN, through a paracrine

action, triggers a number of signaling pathways in cancer cells, which result in the expression of potentially oncogenic, prometastatic and proangiogenic substances, such as prostaglandin E2 (PGE2) and metalloproteinases 2 (MMP-2) and 9 (MMP-9). Mi et al. (2009) demonstrated that ablation of OPN cell surface receptor binding is associated with significant alteration in gene and protein expression critical in key carcinogenesis pathways. Many of these proteins have not been previously associated with OPN. OPN receptor blockage was associated with downregulation of interleukin-10 (IL-10), vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF) and anti-apoptosis signaling with concomitant upregulation of apoptosis, granulocyte-macrophage colony stimulating factor (GM-CSF), anti--proliferative and anti-metastasis signaling pathways [43].

Numerous reports suggest that OPN plays an important role in the process of metastasizing, which requires from proteolytic capabilities of cancer cells enabling the degradation of the extracellular matrix, subsequent migration, proliferation, and angiogenesis in the metastatic site. OPN is involved in all of these processes by binding to integrins in the basal membrane, and on the surface of leukocytes. Integrins mediate several processes leading to the formation of metastases, such as chemotaxis, phagocytosis, adhesion, migration, and proliferation of lymphocytes [44, 45]. Based on protein sequence information, research into cell surface receptors focused on Arg-Gly-Asp (RGD)-dependent interactions of OPN, and these efforts led to the identification of $\alpha_v\beta_3$, $\alpha_v\beta_1$, and $\alpha_v\beta_5$ integrins as OPN receptors. In addition, non-RGD-dependent interactions have since been described. The Leu-Pro-Val (LPV) containing domain of OPN is a site of interaction with α4β1, and a cryptic, Ser-Val-Val-Tyr-Glu-Leu-Arg (SVVYGLR) domain that interacts with α9β1 following exposure by thrombin cleavage of intact human OPN has been identified. The conservation of the thrombin cleavage site in all the OPN sequences that have been determined suggests that cleavage is important in some necessary physiological process. It was discovered that for some cells of hematopoietic origin cleavage of OPN by thrombin enhanced its ability to promote adhesion, confirming earlier reports that for some cell types thrombin-cleaved OPN supported adhesion, spreading, and migration better than the intact molecule. OPN can also enhance cell survival and modulate phosphorylation of intracellular signal transduction intermediates such as focal adhesion kinase, paxillin, and Src [46, 47]. The binding to the integrins is also significant in the metastasis formation process in breast and prostate cancer [48, 49]. Similarly, the binding of OPN to the CD44 receptor may also promote the process of metastasizing by inducing migration and invasion of cancer cells [50]. Some studies suggest that cell migration induced by OPN results from the stimulation of urokinase plasminogen activator (uPA) — a proteolytic enzyme, which results in an increased proteolytic activity [51]. Following migration, cancer cells start to invade and proliferate in distant organs — this is stimulated by OPN that enhances cell proliferation indirectly through VEGF. Tumor growth requires a rich vascular supply, and it has been shown that OPN plays an important role in this process [52].

Chakraborty et al. (2008) demonstrated that OPN, through an autocrine and paracrine action, induces the expression of VEGF that results in angiogenesis [53]. Numerous studies confirm a correlation between the time to recurrence or mortality rate and the serum concentration of OPN in various cancers, such as breast cancer, gastric cancer, hepatocellular cancer, hormone-dependent prostate cancer, and cervical cancer — the greater was the OPN serum concentration, the shorter was the recurrence and survival time. In the analyzed studies, the expression of OPN correlated with lymph node metastasis and advanced TNM stage and histologic grade. Elevated OPN expression levels also correlated with tumor recurrence or metastasis. Overall, the expression of OPN was an independent prognostic factor for both disease-free and overall survival of breast cancer patients [54]. OPN levels was also increased in patients with local recurrence, who were treated with primary radiotherapy for locally advanced head and neck squamous-cell carcinoma. Monitoring of increased levels of postoperative serum OPN concentration has also been proposed for assessing treatment response and tumor recurrence after resection of hepatitis B-related hepatocellular carcinoma. In hepatocellular carcinoma tissues, OPN levels were found to be significantly higher in recurrent tumor tissues compared to non-recurrent tissues. Also patients with higher OPN serum levels had significantly shorter median survival time and recurrence time compared to the ones with lower OPN levels [55]. Moreover, there is an association between OPN concentration and the stage of breast cancer, non-small-cell lung cancer, gastric cancer, hepatic cancer, prostate cancer, and melanoma [44]. In non-small-cell lung cancer and prostate cancer, the concentration of OPN is correlated with the presence of metastases in bones [45] and the disease stage. In the head and neck cancers, OPN concentration is associated with tissue hypoxia [48], which leads to disease recurrence [49] — the expression of the OPN gene is correlated with a high expression of the VHL gene that regulates hypoxia [50]. Certainly it appears that OPN concentration level has significant correlative relationship with aggressive disease and poor prognosis. However, OPN is not a universal marker for tumors and metastasis. It is upregulated in response to stress and inflammation and hence its use is clinically not favored [56].

Takafuji et al. (2007) demonstrated that OPN was present in different molecular forms in the human hepatic carcinoma samples [57]. The smallest form — OPN 5 kDa correlated with cancer stage and induced cancer cell invasion by promoting

binding to the CD44 receptor. The authors suggested that the increased expression of OPN-c during the process of metastasizing might result from the fact that it contains the OPN 5 kDa fragment. Therefore, OPN 5 kDa might be crucial in the neoplastic transformation and become a potential treatment target [57].

CD44 plays a significant role in the tumor initiation and progression in colorectal cancer, but how the molecule benefits cancer cells from the tumor microenvironment, especially tumor-associated macrophages, remains poorly defined. OPN was highly expressed in hepatic metastasis from colorectal cancer compared to primary colorectal tissue and adjacent normal mucosa. Exogenous expression of OPN in colorectal cells increased heterotypic adhesion with endothelial cells [58]. What is more, OPN induced by macrophages contributes to metachronous liver metastasis in colorectal cancer [59]. A recent study reports on the reciprocal interactions between tumor-associated macrophages and CD44-positive colorectal cancer cells via OPN/CD44 interaction. Rao et al. (2013) found that macrophages, when cultured with CD44-positive colorectal cancer cells, were able to produce higher levels of OPN, which in turn facilitated the tumorigenicity and clonogenicity of the colorectal cancer. The knockdown of CD44, such as treatment with CD44 blocking antibodies, attenuated OPN secretion. OPN, through binding to its receptor CD44, activated c--jun-NH(2)-kinase signaling and caused the clonogenicity of colorectal cancer cells. What is more, tissue microarray data showed that OPN expression, in combination with CD44v6, has a negative correlation with colorectal cancer patient survival. These results suggest that the OPN-CD44 interaction is important for colorectal cancer progression and could suit as a potential therapeutic target for the treatment of colorectal cancer [60].

Regarding angiogenesis, studies have shown that OPN expression is strongly correlated with tumor microvessel density, and the OPN-derived peptide SVVYGLR potentiates tube formation in three-dimensional collagen gels [61]. This increase in angiogenesis may be through PI3K/AKT- and ERK--mediated pathways with VEGF acting as a positive feedback signal, as the inhibition of angiogenesis was stronger with an OPN-antibody than with a VEGF-antibody, or PI3K and ERK inhibitor [62]. Furthermore, Tang et al. (2007) showed that the proliferation, migration, and tube formation of human umbilical vein endothelial cells was reduced following OPN knockdown in gastric cancer cells [63]. Additional studies have also shown that the expression of some tumor-associated genes that play an important role in metastatic processes, such as VEGF, MMP-2, MMP-9, and μPA, are closely associated with OPN [64–66], which is likely one of numerous prognostic factors related to colorectal cancer metastasis [67].

Liu et al. (2010) have reported that OPN induces expression of MMP-2 and MMP-9 via NF-κB-mediated signaling pathways in prostate cancer [66]. In addition, the expression of MMP-2 and MMP-9 is correlated with angiogenesis and metastasis of colorectal cancer [68]. Thus, OPN downregulation could inhibit not only the MMPs, but also VEGF and μPA expression in colon cancer cells, which might lead to decrease invasiveness and angiogenesis capacity of colorectal cancer cells.

The fact that overexpression of OPN is associated with metastasis was also proved in the study by Huang et al. (2012). Results of this study showed the high influence of OPN overexpression on liver metastasis formation in colorectal cancer patients. The liver is the primary extra-colonic site for colorectal cancer metastasis and represents the most frequent location and clinical presentation for recurrent disease in patients who failed locoregional therapy. Furthermore, liver metastasis is a main cause of mortality from colorectal cancer. In the study by Huang et al. (2012) OPN mRNA was examined in tissues from colorectal cancer, adjacent normal mucosa, and liver metastasis. The OPN and its receptor expression were detected by using an immunohistochemical methods. The expression of OPN mRNA in tumor tissues was significantly correlated with the colorectal cancer stages. OPN expression was also detected in normal hepatocytes surrounding colorectal cancer metastasis. Both known so far OPN receptors, integrin α_v and CD44v6 proteins, were strongly expressed in hepatocytes from normal liver. Colorectal cancer cells with intensified OPN expression exhibited increased heterotypic adhesion with endothelial cells and weakened intercellular communication. Because of OPN, homotypic adhesion ability is weakened in colorectal cancer cells and the cells to depart from primary site more easily, and enter the blood circulation. However, these cells are more easily to invade the extracellular matrix when heterotypic adhesion is enhanced. These two treads are important to metastasis in malignant cancer [69].

Diagnostic role of osteopontin

Visintin et al. (2008) report that OPN, combined with other markers such as leptin, prolactin, insulin-like growth factor 2 (IGF-2), macrophage migration inhibitory factor (MIF), and cancer antigen 125 (CA-125), enhances diagnostic sensitivity to 95% and diagnostic specificity to 99% in ovarian cancer [70]. None of the biomarkers by themselves were good enough to differentiate healthy versus cancer cells. However, the combination of the six markers, and OPN among them, provided a better differentiation than only CA-125 assessment (sensitivity 72% at specificity 95%). OPN concentration in ovarian cancer patients was indeed significantly greater than in healthy controls. In patients who underwent chemotherapy, an increase in OPN concentration precedes clinical recurrence even when the concentration of CA-125 remains within the normal range [70].

Also in skin cancer, an increased OPN tissue expression was determined by immunohistochemistry in squamous

cell carcinoma (SCC) and in precancerous cells and compared to the healthy cells, although these findings were not unambiguous [71, 72].

The greatest number of reports links OPN to the process of metastasizing, which is reflected by a wide interest in the molecular mechanisms of OPN action. The molecular targets could enable the interference with the process of metastasizing and thereby lead to improved outcomes. It is astonishing how greatly is OPN's structure modified posttranslational. This leads to the development of different OPN forms characterized by various molecular mass that might play different roles in the process of metastasizing. The most coherent classification describes three different particles — OPN-a, OPN-b, and OPN-c [73]. In an invasive breast cancer cell line, only OPN-c has been detected, whereas it was not present in a non-invasive cell line or in healthy tissue. Moreover, OPN-c concentration is associated with cancer stage, and this form of OPN might be a marker of metastatic disease in various cancers [73].

The results of the study by Ting-Ting et al. (2014) show that compared with peri-carcinomatous tissues, abnormal expression of OPN occurs in the non-small-cell lung cancer (NSCLC) patient's tissues, which indicates that OPN may participate in the development of NSCLC. Moderately positive expression of OPN was found in 34.6% and strong expression in 47.5% of the NSCLCs. OPN expression in carcinomas was higher than in peri-carcinomatous tissues ($p < 0.05$). While no obvious association was observed with NSCLC patient age, gender, maximum diameter of the tumor and pathological type, OPN expression was more commonly detected in poorly differentiated carcinomas and these with lymph node metastasis, as well as at advanced clinical stage ($p < 0.05$). The expression of OPN in patients with regional lymph node metastasis was 93.0% and in patients without regional lymph node metastasis was 75.3%. Positive expression rates of patients of high, medium and low differentiation were 53.1%, 88.4% and 95.6% respectively. OPN expression was also positively correlated with micro-vascular density (MVD), namely, MVD count increased with high expression of OPN ($p \leq 0.001$) [74].

These results show that abnormal expression of OPN is closely related with outside invasion and distant metastasis, but does not enhance the continued proliferation ability of tumor in a certain fixed position and affect the formation of ecological niche before metastasis. This phenomenon may result from the fact that OPN promotes the neovascularization and lymphatic vessel, as well as the increase of vascular permeability and eventually causes lymphatic and blood metastasis.

The tumor MVD is used to suggest the quantity of neovascularization and is a quantitative indicator of degree of tumor neovascularization, suggests the tumor recurrence, metastasis potential and long-term survival. The correlative analysis presented that OPN expression level was positively correlated with MVD expression in NSCLC ($p < 0.01$), which showed that OPN plays a very significant role in neovascularization and may provide material basis for the vascularization of tumor in the process of growth and metastasis of tumor, while vascularization further provides necessary pathways for the tumor cell metastasis [74].

OPN also seems to play an important multidirectional role in colorectal cancer, which is one of the most frequent malignant neoplasms worldwide and also one of the leading causes of cancer-related mortality. Up to now, no biomarker has been used to predict the prognosis and surveillance of patients with colorectal cancer. Recently, the association between OPN overexpression and the prognosis of colorectal cancer was investigated widely, but the results were inconsistent. Therefore, the aim of meta-analysis by Zhao et al. (2015) was to assess the prognostic effect of OPN in patients with colorectal cancer. A total of 15 studies showed that high OPN expression was significantly associated with high tumor grade, lymph node metastasis and tumor distant metastasis. What is more, high OPN expression was significantly associated with the 2-year, 3-year, 5-year survival rate and overall survival, respectively, although the cut-off value of high OPN should be further studied. What is really significant, the ELISA blood assay may be the best way for OPN detection. OPN can be used to evaluate clinicopathology of tumor preoperatively and for the surveillance of tumor recurrence postoperatively. All these results indicate that OPN may serve as a prognostic biomarker and as a potential therapeutic target for colorectal cancer [75].

The study by Wu et al. (2014) was also focused on the role of OPN in colorectal cancer biology. It was proved that knockdown of OPN gene expression suppresses colorectal cancer cell growth, adherence, invasion and expression of angiogenic factors [76].

Can osteopontin influence stem cells biology?

Hepatocellular carcinoma (HCC) is the fifth most prevalent cancer worldwide and as such presents a major global health burden. Metastasis, tumor recurrence and chemo-resistance are among the leading causes of mortality of patients with HCC. There is an increasing evidence that suggest the HCCs are sustained by a distant subpopulation of self-renewing cells know as cancer stem cells. Cao et al. (2015) demonstrated that the level of OPN in tumor cells of the edge of bulk tumors was significantly correlated with the clinical prognosis of patients with HCC. OPN was highly expressed in side population fractions of HCC cell lines, as well as in dormant cells, spheroids and chemo-resistant cancer cells, all of which are considered as having stemness-like cellular features. Depletion of OPN in hepatocellular carcinoma resulted in a reduction in the proportion of side population fractions, formation of hepato-spheroids,

expression of stem-cell-associated genes and decreased tumorigenicity. Mechanistically, OPN was demonstrated to bind to integrin $\alpha_v\beta_3$ and activate the transcription factor NF-κB, which resulted in upregulation of *HIF-1α* transcription and its downstream gene, *BMI1*, to mediate maintenance of the stemness-like phenotype. Suppression of the $\alpha_v\beta_3$–NF-κB–HIF-1α pathway decreased OPN-mediated self-renewal capabilities. Levels of OPN were significantly correlated with HIF-1α protein levels in HCC tumor tissue samples [80]. These results indicate OPN might promote a cancer stem cell-like phenotype via the $\alpha_v\beta_3$–NF-κB–HIF-1α pathway. Findings by Cao et al. (2015) offer strong support for OPN requirement in maintaining stem-like properties in HCC cells [77].

A poor cancer outcome can be associated with osteopontin overexpression

Recent studies showed high expression of OPN in NSCLC and its association with poor patient outcome. Serum OPN levels increased according to tumor pT classification and tumor size, and patients with OPN-expressing tumors had higher serum OPN levels than patients with OPN-negative tumors. Rud et al. (2013) showed that tumor OPN expression is a strong predictor of poor prognosis, and multivariate analysis confirmed OPN as an independent prognostic factor. The finding that patients with OPN-expressing tumors have worse relapse free and overall survival than patients with OPN-negative tumors indicates that OPN has the potential to be used as a prognostic biomarker in NSCLC. The finding that tumor OPN expression, but not serum OPN level, was associated with poor survival may be clarified by the multi-functionality of OPN [78]. The majority of OPN activities ascribed the interaction between secreted OPN and its receptors on target cells, however OPN is also found intracellularly and the non-secreted form is involved in cellular processes such as motility and migration [79, 80]. Results of the study by Rud et al. (2013) indicated that the intracellular levels of OPN are more significant than the secreted circulating levels in NSCLC [78]. Furthermore, variations in serum OPN measurements may occur due to proteolytic cleavage of circulating OPN [81]. Ultimately, OPN has an important role in inflammation and wound healing, and therefore other systemic sources than the tumor itself may affect OPN levels in the circulation.

The finding that OPN-c expression is a predictor of patient survival in breast cancer, which does not correlate with cyclins, receptor status or family predisposition, adds to previous reports, that this splice form is an independent biomarker. OPN-c showed no association with ER, PR or HER2, however it was highly expressed in triple negative breast cancer. The multivariate analysis of this study corroborates the prior observation that the diagnostic and prognostic values may be enhanced by combining OPN-c with the receptor status of the cancer. OPN-c in breast cancers was reported to correlate with relapse or poor survival [82]. According to all these conclusions, the addition of OPN-c immunohistochemistry to standard pathology work-ups may have prognostic benefit in early breast cancer diagnosis.

Osteopontin promotes resistance to chemotherapy

Multidrug resistance is a major cause of chemotherapy failure. Recent studies indicate that drug resistance can be rapidly induced by some factors, such as cytokines, chemokines, growth factors, and cell adhesion factors in the tumor microenvironment. As indicated previously, OPN has a functional arginine-glycine-aspartic acid domain for binding to integrin. I-Shan et al. (2013) found OPN expression to be upregulated by hypoxic condition in prostatic tumor cells. OPN increased the mRNA and protein expression level of p-glycoprotein (P-gp), a subfamily of ATP-binding cassette transporter in a concentration and time-dependent manner. The increase in P-gp expression by OPN was mediated by binding to $\alpha_v\beta_3$ integrin. Daunomycin, a chemotherapeutic agent with autofluorescence, was used to evaluate the pump activity, and OPN increased the drug pumping-out activity. OPN inhibited daunomycin-induced cell death, which was antagonized by $\alpha_v\beta_3$ monoclonal antibody. Long-term treatment with daunomycin further enhanced the expression of OPN. Knockdown of endogenous OPN potentiated the daunomycin-induced apoptosis of prostatic cancer cells. Moreover, knockdown of OPN enhanced cell death caused by other drugs, including paclitaxel, doxorubicin, actinomycin-D, and rapamycin, which are also P-gp substrates. These results indicate that OPN is a potential therapeutic target for cancer therapy to reduce drug resistance in sensitive tumors [83].

One protein, thousands of interactions

It was proved by Stemberger et al. (2014) that OPN is associated with decreased apoptosis in lung adenocarcinoma. The level of OPN expression in lung adenociarcinoma was associated with decreased apoptotic activity of tumor cells ($p = 0.006$), and correlated with α_v integrin expression ($p = 0.048$), particularly in low stage tumors ($p = 0.013$). Study showed that prolonged tumor cell survival in lung adenocarcinoma due to OPN and α_v integrin overexpression may have an impact on tumor progression and resistance to therapy [84]. In human lung cancer cells, OPN knockdown suppressed lung cancer cell invasion and metastasis and also induced autophagy and abrogated the radio-resistance of the cancer cells [65]. While OPN concentrations were significantly higher in lung cancer patients compared to controls, a negative correlation was detected between OPN and body mass index (BMI), suggesting that in addition to being an indicator of systemic inflammation in lung cancer patients, OPN may also be an indicator of advancement of the disease and cachexia [86].

Although OPN has been known as a marker for cancer progression, the elevated production of this cytokine is not specific only for cancer. Zduniak et al. (2015) have identified recently the splice variant OPN-c as being absent from healthy tissue but associated with about 75% of breast cancer cases. Scientists found that high staining intensity of nuclear OPN-c was strongly associated with mortality in patients with early breast cancer. Cytosolic staining for exon 4, reflective of OPN-a and -b variants also predicted poor outcome. By contrast, total OPN did not correlate with prognosis. These diverse assessments of OPN also did not correlate with each other, suggesting distinct expression patterns for the variant forms. Consistent with its role in tumor progression, not tumor initiation, OPN-c is not correlated with proliferation markers (Ki-67, cyclin A, cyclin B, cyclin E and cyclin D), neither is it correlated with ER, PR or HER2 [82].

Conclusions

There is an increasing number of reports on the role of OPN in various medical fields such as oncology, allergology, nephrology, and cardiology [87]. Similarly, there are plenty of studies that point to a role of OPN in the progression in various cancers [88]. Scientists suggest that the knowledge of the processes in which OPN is involved may help in the diagnosis and prognosis of cancer metastasis, as well as in cancer staging and treatment monitoring [89].

Conflict of interest: none declared

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