Full-length osteopontin and its splice variants as modulators of chemoresistance and radioresistance (Review)

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Abstract. Osteopontin (OPN) is a matricellular phosphoglycoprotein overexpressed in several tumor types and can activate several aspects of cancer progression in solid and non-solid tumors. In the present review, the roles of OPN in mediating resistance to chemotherapy and radiotherapy and their main associated signaling pathways were summarized and discussed. Furthermore, it was detailed how OPN expression may be able to modulate resistance to these therapies by controlling epithelial cell plasticity, stemness potential and cell survival. Based on these data, the use of OPN and associated signaling was then proposed as potential molecular targets in order to sensitize resistant cells to main current therapeutic approaches. Finally, based on experimental evidence obtained by our group, the importance of investigating the specific roles OPN splicing isoforms have and how their properties may specifically control resistance to therapy was highlighted. These data elucidate a better understanding of how total OPN and their splicing isoforms, as well as their associated signaling, may contribute to main aspects of chemoresistance and radioresistance, such as those controlling cell survival, apoptosis, autophagy, stemness, epithelial cell plasticity and associated cell receptors.

Contents

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1. Introduction

Cancer therapy resistance is mediated by several mechanisms, including intrinsic and extrinsic factors, and those originating from the tumor microenvironment (TME) (1). Most of the widely used chemotherapeutic agents and γ-radiation utilize apoptosis or autophagy as common death pathways. Thus, a better understanding of the molecular mechanisms behind tumor biology and cancer therapy resistance is a mandatory step to propose novel approaches aiming to bypass chemotherapy and radiotherapy resistance and associated gene products. Among several markers associated with response to therapy, osteopontin (OPN) has been identified as a key molecule (2,3).

OPN is a multi-functional chemokine-like matricellular phosphoglycoprotein. Depending on its intracellular or extracellular localization, OPN is involved in a series of physiological roles, including inflammation, cell adhesion and migration, differentiation, cell survival and apoptosis, as well as regulation of bone matrix mineralization. These diverse biological roles are partly due to its capacity to interact with several molecules, including cell surface receptors, such as integrin and cluster of differentiation (CD44), intracellular signaling molecules, calcium and heparin. OPN is produced by distinct cell types, such as epithelial, stromal, immune system, bone and endothelial cells (4). High OPN expression has also been detected in adipose tissues and body fluids (4).

In multiple cancer types, OPN expression is upregulated (3). In tumors, OPN regulates tumorigenesis, tumor progression and metastasis formation (5), by activating cell migration, inhibiting apoptosis (6,7), stimulating angiogenesis (8) and metabolism (9), and modulating the tumor microenvironment (10) and the immune system (11). Notably, OPN can also promote cell survival by negatively regulating apoptosis in response to stress conditions, including exposure to anticancer agents effect (12). OPN performs these roles by binding to cell surface receptors, including integrin and CD44 cell receptors (13).

Several aberrantly activated signaling pathways can activate OPN expression in cancer cells, such as activator protein-1, mycA, phosphatidylinositol 3 kinase (PI3K), serine/threonine kinase (AKT) and nuclear factor-κB (NF-κB) (14, 15). Notably, OPN has been described as a key modulator of cancer hallmarks, by regulating the main aspects of tumor progression in several tumor models (16,17). OPN is able to promote tumor cell migration by interacting with integrin receptors, especially αvβ3 integrin, stimulating cell adhesion and tumor cell migration properties (3). It has been demonstrated previously that OPN downregulation in breast cancer cell lines inhibits OPN interaction with αvβ3 integrin, thereby impairing cell migration, invasion and apoptosis (18). It was demonstrated that these effects have been mediated by PI3K/AKT/mechanistic target of rapamycin (mTOR) signaling, promoting the upregulation of light chain 3 and beclin-3, then favoring apoptotic cell death, while inhibiting aggressive phenotype of these breast cancer cells. It has also been demonstrated that alteration of OPN expression levels may influence tumor growth, migration and cell cycle in human nasopharyngeal CNE-2 carcinoma cell lines (18). When downregulating OPN expression, diminished levels of matrix metalloproteinase MMP-2 and MMP-9 have been observed, evidencing that OPN may also induce MMP expression levels through the activation of NF-κB signaling in this tumor model (19). OPN expression levels can also modulate mitochondrial mediated apoptotic cell death, involving cytochrome *c*, apoptotic protease activating factor 1, cleaved caspase-3 and B cell lymphoma (Bcl)-2/Bcl-2-associated X protein, resulting in lower expression levels of proteins associated with cell invasion, such as MMP-2 and urokinase-type plasminogen activator (uPA) (20). Then, downregulation of OPN expression levels can promote apoptotic cell death and cell invasion properties in a mitochondrial-dependent pathway (20). OPN can also promote tumorigenesis and tumor progression by evading apoptotic cell death, mainly by interacting with CD44 cell receptors (21). Furthermore, OPN interactions with immune and inflammatory cells from the TME perform essential roles on tumor development and progression. Besides being produced by tumor cells, OPN can also be secreted by stromal and infiltrating inflammatory cells that can affect the TME and its corresponding cell roles. Stromal fibroblasts can also be influenced by OPN (22,23). Given its roles in angiogenesis, extracellular matrix remodeling and metastasis, their presence and influence by OPN can also favor tumor growth (22,23). It has been reported that when interacting with α9β1, OPN activates p38 and extracellular signal-regulated kinase (ERK) signaling, which then can promote the expression of cyclooxygenase-2 and prostaglandin E (PGE), favoring melanoma tumor cell migration (24). In addition, macrophages from the TME, when activated by OPN can promote angiogenesis via PGE2 and stimulate the expression of MMP-9, consequently promoting tumor progression in this tumor model (24). When recruiting macrophages to the tumor inflammatory environment, OPN can also stimulate tumorigenesis (25).

OPN also induces the expression and activity of MMPs, which can contribute to tumor metastasis by degrading the extracellular matrix (ECM) and promoting cell invasion. Conversely, OPN biological activity can also be modulated by MMP-induced cleavage (26). It is well-known that OPN stimulates tumor cell invasion and migration possibly by inhibiting apoptotic cell death and by regulating the activity of MMP-2 and MMP-9 that degrade the ECM (27). OPN can stimulate the activity of MMP-9, modulating multiple signaling pathways, such as focal adhesion kinase (FAK), ERK and NF-κB, which then can regulate cytoskeleton architecture, cell growth, motility and extracellular matrix (ECM) (28,29). By activating PI3K/AKT signaling, OPN can similarly regulate hypoxia-inducible factor (HIF)-1α expression via αvβ3 integrin interaction and promoting ECM degradation through uPA and MMP-9, further mediating metastasis formation in ovarian cancer cells (30).

Also in the context of OPN roles on modulating the TME and the immune system, OPN has been described as a multifactorial cytokine activated by T lymphocytes, monocytes, macrophages, epithelial cells, fibroblasts and a promoter of cell-mediated immune responses (15,28).

Similarly, OPN is a typical angiogenesis stimulating factor, sustaining tumor progression and metastatic growth. The role of OPN in angiogenesis is mainly associated with OPN interaction with αvβ3 integrin, a central angiogenesis marker (31), but is also associated with several other factors, including vascular endothelial growth factor (VEGF) (32,33). OPN interactions with VEGF are also mediated by aberrant signaling pathways, such as PI3K/AKT and ERK1/ERK2 (32,33). It has also been demonstrated in acute leukemia that the expression of OPN and VEGF are strongly correlated with the occurrence and development of this non-solid tumor. In this model, OPN can regulate VEGF expression and promote angiogenesis besides favoring disease progression (31). It was also found that OPN interaction with αvβ3 activates signaling pathways, such as breast tumor kinase/NF-κB/activating transcription factor (TF)-4, promoting cell migration, tumor growth, endothelial cell migration and angiogenesis (34). Our group also demonstrated that OPNb and OPNc splicing isoforms favored tumor growth, cell proliferation, invasion and migration by modulating VEGF, MMP-2 and MMP-9 expression levels through PI3K signaling (35).

It has also been demonstrated that OPN can modulate metabolism by signaling through the activation of oxidoreductase gene expression, associated with the mitochondrial respiratory chain, the hexose monophosphate shunt or the regulation of the hexose monophosphate shunt (9). Furthermore, it has been reported that OPN can disrupt liver cholesterol metabolism (36).

The majority of the data regarding the role of OPN in cancer cell refers to total OPN, which includes the sum of all OPN isoforms, including those generated by alternative splice, post-translational modifications and alternative translation (17,37).

OPN primary transcript suffers alternative splicing, generating at least five splice variants, named OPNa (which contain all coding exons), OPNb (exon 5 is deleted), OPNc (exon 4 is deleted), OPN4 (in which both exon 4 and exon 5 are deleted) and OPN5 (which contains an additional exon originated from inclusion of a region from intron 3; Fig. 1). OPN splicing isoforms (OPN-SI) are aberrantly expressed in cancer cells (17). Particularly, the expression and functional roles of OPNa, OPNb and OPNc have been broadly studied in distinct tumor models, in which they were demonstrated

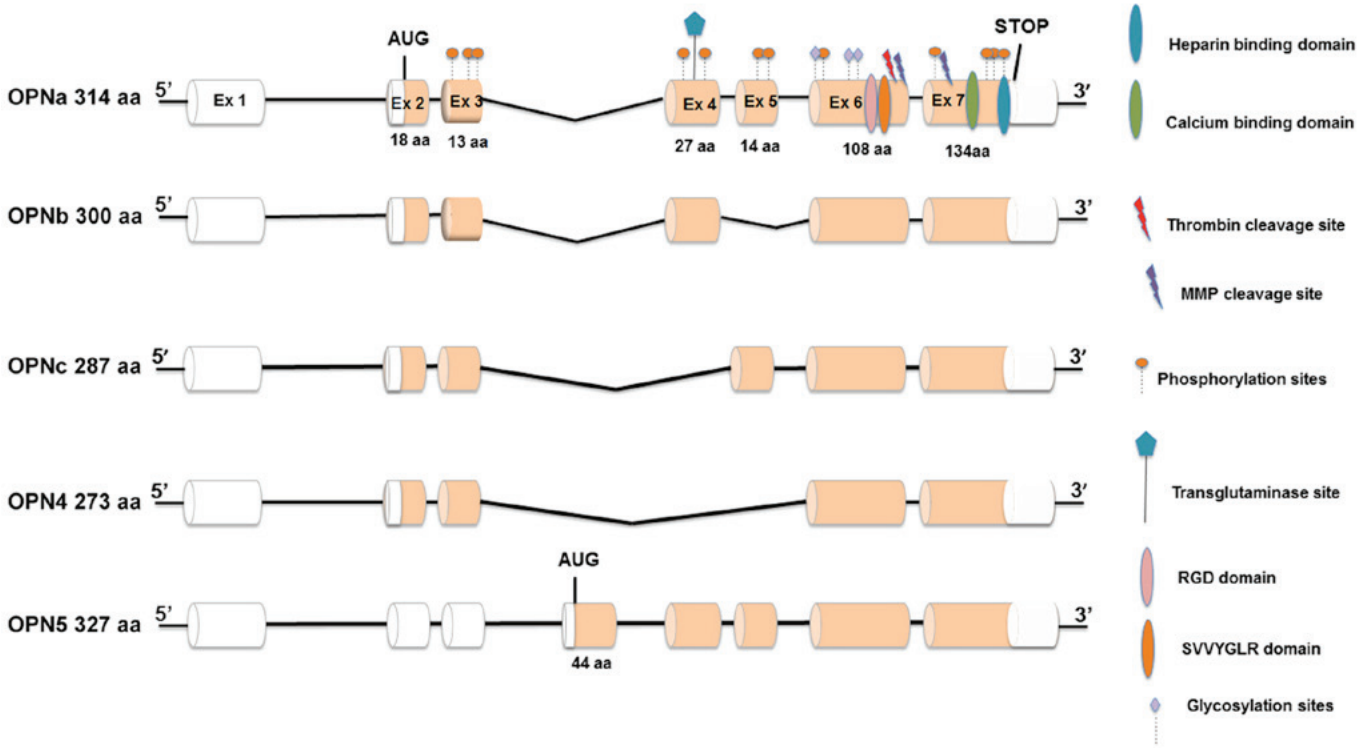

Figure 1. Structure of OPN isoforms. Exonic regions represented by white cylinders correspond to 5' and 3' untranslated regions. Each translated Ex is represented by orange cylinders. Ex total lengths are also presented. OPN functional domains are also presented above each Ex, such as RGD, SVVYGLR and calcium binding domain, as well as post-translational modifications, such as phosphorylation and glycosylation sites. Also presented are protein cleavage sites for thrombin and MMPs. The initiation translation codon (AUG) and the stop codon are also represented. OPNa, the full length isoform, contains Ex 2-7, whereas OPNb and OPNc lack Ex 5 and 4, respectively. Ex 4 and 5 are deleted in the OPN4 isoform, whereas OPN5 contains an extra Ex resulting from an inclusion of an intronic region located between Ex 3 and 4, corresponding with the longer isoform. Total length of each isoform of each isoform is also presented. OPN, osteopontin; aa, amino acids; MMP, metalloproteases; Ex, exon.

to present tissue and tumor specific roles (17,37). Notably, the same OPN-SI might activate or inhibit tumor progression, depending on the tumor type (17,37). However, data regarding the expression and functional roles of OPN4 and OPN5 in tumor cells are scarce. One previous report described that these recently described OPN-SI are frequently co-expressed in esophageal carcinoma tissues (38), but functional studies evaluating these splice variants are still lacking.

In the present review, among the broadly known OPN roles on activating tumor progression, additional features involved in tumor resistance are highlighted. These typically include OPN functions on modulating cell survival, apoptosis and autophagy, as well as cell plasticity and sustaining stem-like properties of cancer cells (39), which are key factors associated with tumor resistance.

A molecular mechanism frequently associated with failure in the treatment of malignant carcinomas is the biological reprogramming of epithelial cells called epithelial mesenchymal plasticity. More recently, it has been defined as a dynamic process implicated in epithelial-mesenchymal transition (EMT) and its reverse program, mesenchymal-epithelial transition (MET) or intermediate phenotypes, known as partial or intermediate EMT (40,41). EMT renders cancer cells the ability to lose epithelial traits, while gaining mesenchymal features. During EMT, cells also acquire stem cell-like properties and are able to disseminate and colonize to distant organ sites, where they may exhibit elevated resistance to cancer therapies (42,43). Aberrant activation of oncogenic signaling pathways, including Wnt/β-catenin, hedgehog, Notch , PI3K-AKT, tumor necrosis factor-α and transforming growth factor (TGF)-β has a critical role in EMT (44) and occurs during acquisition of EMT phenotype and resistance to therapy. Furthermore, induction of resistance is also mediated by several genes regulated by the TF NF-κB, including Bcl-2, Bcl-xL, X-linked inhibitor of apoptosis (XIAP), survivin and AKT, which have been reported to mediate chemoresistance and radioresistance in numerous types of tumor cells (45).

In the present report, current knowledge regarding the OPN roles on mediating chemoresistance and radioresistance were reviewed, which are the primary cancer treatment approaches that are currently used. The distinct mechanisms by which OPN can promote resistance to specific drugs and their associated signaling pathways are also explored. Based on these data, putative treatment approaches targeting OPN that have been proposed to overcome resistance or inhibit tumor progression, and the particular contribution of OPN splice variants on the resistant phenotype are then discussed.

2. OPN and resistance to chemotherapy

OPN is able to mediate resistance to distinct chemotherapeutic drugs and in several cancer types. Table I summarizes these data and the corresponding signaling pathways and molecular mechanisms involved.

In non-solid tumors, such as leukemia, OPN has been reported to mediate resistance to parthenolide and sorafenib.

Table I. OPN roles in mediating chemoresistance and radioresistance.

Author, year	Drug or therapy	Tumor type	Biological material	Main findings	Signaling pathways involved	Refs.
Zhang *et al*, 2014	PTL	AML	AML cells resistant to DNR	OPN promotes resistance to PTL in $CD34^+/CD123^+$ leukemia stem cell population resistant to DNR	PTL treatment downregulates OPN, and inhibits AKT, mTOR, β-catenin and PTEN	(18)
Zhang *et al*, 2010	Sorafenib	AML	TCGA data; MV4-11 human acute leukemia cell line (*FLT3-ITD* mutation)	OPN binds to integrin αvβ3 and decrease sorafenib sensitivity in FLT3-ITD mutated AML cells	Sorafenib sensitivity involves OPN/αvβ3 integrin/PI3K/ AKT/GSK3β/β-catenin pathway	(20)
Gu *et al*, 2009	CDDP	Human small cell lung cancer	Stable OPN overexpression in SBC-3 cells (SBC-3/OPN)	Intracellular OPN mediates resistance to CDDP in SBC-3 cells	OPN induces anti-apoptotic Bcl-2 while blocking caspase-3 and -9	(5)
Polyak *et al*, 2009	CDDP	OSCC	SAS cells	OPN overexpression promotes resistance to CDDP	Not investigated	(23)
Luo *et al*, 2015	5-FU	OSCC	SAS cells overexpressing OPN	OPN-αvβ3 integrin axis mediates resistance to 5-FU	OPN-mediated resistance involves αvβ3 axis	(50)
Ng *et al*, 2015	Oxaliplatin	Colorectal cancer	DLD1 cells overexpressing OPN	OPN expression and chemoresistance in patients treated with oxaliplatin	OPN enhances stemness of cancer cells through CD44v6, HGF and SDF-1	(51)
Lin *et al*, 2015	Temozolomide and CDDP	Glioma	U251 human glioma cells	OPN mediates resistance to temozolomide and CDDP	OPN-mediated resistance involves NF-κB/Bcl-2 pathway	(52)
Shao *et al*, 2017	CTX	Breast cancer	MDA-MB-231	OPN knockdown sensitizes cells to CTX	OPN-mediated resistance involves p38 MAPK pathway	(26)
Guarneri *et al*, 2017	Vinorelbin, etoposide and gemcitabine	Malignant pleural mesothelioma	ACC-MESO-1/OPN cells overexpressing OPN	OPN is involved in multi drug resistance by enhancing the CD44 binding to HA	CD44 and HA binding and activation of PI3K signaling	(27)
Wang *et al*, 2011	Radiotherapy	Lung cancer	NSCLC cell lines and xenograft tumors	OPN and EGFR pathway, have been associated with radiation resistance in lung cancer cells	Radiation resistance in tumors harboring KRAS mutations involves MLCC and CSC-like phenotypes	(31)
Song *et al*, 2008	Radiotherapy	Lung cancer	A549 lung cancer cells	Beclin1-induced autophagy abrogates radioresistance by suppressing OPN	Radioresistance is mediated by inhibition of Beclin1-induced autophagy	(30)
Castello *et al*, 2017	Radiotherapy	Cervical cancer	Radiation resistant and sensitive LACSCC paraffin tissues	OPN and E-cadherin aberrant expression indicates radiation resistance	OPN-mediated EMT mediates radioresistance	(32)

OPN, osteopontin; 5-FU, fluorouracil; CDDP, cisplatin; PTL, parthenolide; OSCC, oral squamous cell carcinoma, Bcl-2, B-cell lymphoma-2; DNR, daunorubicin; AML, acute myeloid leukemia; PTEN, phosphatase and tensin homolog; HGF, hepatocyte growth factor; SDF-1, stromal cell-derived factor-1; CD, cluster of differentiation; CD44v6, variant isoform of CD44; CTX, cyclophosphamide; MLCC, mitosis-like condensed chromatin; LACSCC, locally advanced cervical squamous cell carcinoma; AKT, protein kinase B; mTOR, mechanistic target of rapamycin; TCGA, The Cancer Genome Atlas; GSK3β, glycogen synthase kinase 3β; NF-κB; nuclear factor-κB; MAPK, mitogen-activated protein kinase; HA, hyaluronan; PI3K, phosphoinositide 3-kinase; NSCLC, non-small cell lung carcinoma; EGFR, epidermal growth factor receptor; CSC, cancer stem cell; EMT, epithelial-mesenchymal transition.

The natural compound parthenolide was demonstrated to induce apoptosis in daunorubicin-resistant stem-like leukemic cells through OPN downregulation and modulation of AKT, mTOR and β-catenin signaling (46). Particularly in acute myeloid leukemia, OPN was demonstrated to be upregulated in leukemic blasts, being correlated with a poor clinical outcome (47). Consistently, OPN promoted resistance to sorafenib by binding to αvβ3 integrin and by inducing β-catenin expression in an AKT and glycogen synthase kinase (GSK)3β-dependent manner (48).

Furthermore, there are several examples in which OPN mediates resistance in solid tumors, most of which focused on the roles of OPN in hepatocellular carcinomas (HCC). In these tumors, high OPN expression level is correlated with increased metastatic potential and resistance to taxanes or cisplatin. In lung cancer cells, intracellular OPN upregulation promotes cisplatin resistance by inducing the expression of anti-apoptotic protein Bcl-2 and by blocking caspase-3 and caspase-9 from activation (5). Also, OPN is able to stimulate HCC cell survival and autophagy, which then can favor stem cell-like properties and the resistant phenotype in response to epirubicin and cisplatin via binding with its receptor integrin αvβ3 and sustaining Forkhead box (Fox)O3a stability (42). It has been proposed that OPN may promote a cancer stem cell (CSC)-like phenotype via the αvβ3-NF-κB-HIF-1α pathway (49).

Other studies using human samples with oral squamous cell carcinoma demonstrated that OPN expression levels are correlated with therapy response and shorter overall survival. OPN upregulation in these tumors promoted resistance to cisplatin and to 5-fluorouracil and also involved the OPN-integrin αvβ3 axis (50). In addition, several reports described the association between upregulated OPN expression and chemoresistance in patients treated with oxaliplatin in colorectal cancer (51), cisplatin in lung cancer (5), temozolomide and cisplatin in glioma (52), cyclophosphamide in breast cancer (53) and vinorelbin, etoposide and gemcitabine in malignant pleural mesothelioma (54). Similarly to other tumor models, resistance to these chemotherapeutic drugs mainly involves the regulated expression of octamer-binding TF 4 and sex determining region Y-box (SOX)2 (modulators of stemness), Bcl-2, caspase-3 and caspase-9 (apoptosis regulators) (5,52), as well as NF-κB, AKT and p38/mitogen-activated protein kinase (MAPK) signaling (49,51). Furthermore, it has been demonstrated in malignant pleural mesothelioma that OPN could regulate chemosensitivity to vinorelbine, etoposide and gencitabin through the alteration of CD44 binding to hyaluronate (HA) (54). HA is a linear glycosaminoglycan that interacts with cell surface receptors, including CD44, facilitating cell adhesion, cell motility, cellular proliferation and tumor progression (54). OPN is strongly involved in multidrug resistance in this tumor by enhancing the CD44 binding to HA and PI3K/AKT signaling, thereby promoting cell survival and chemoresistance (54).

3. OPN and association to radioresistance

In addition to OPN roles in mediating chemoresistance, reports describing the functional association between OPN and radiotherapy resistance are scarce (Table I). However, a number of reports have described the roles of OPN as a marker of response to radiotherapy (55,56). OPN expression, in conjunction with the epidermal growth factor receptor pathway, have been associated with radiation resistance and poor prognosis in lung cancer, particularly in patients presenting tumors with KRAS mutations (57). It has also been reported in lung cancer that OPN is an indicator of resistance to radiotherapy. In a previous study, overexpression of beclin-1 induced cell death by autophagy in human lung cancer cells, reversing radioresistance (58). Radioresistance in these tumors has been associated with a stem cell-like phenotype and invasive potential (57). In cervical cancer, high OPN and low E-cadherin expression levels correlate with a radiation-resistant phenotype (59), further implicating OPN as a key molecule in the interface of radioresistance and cellular plasticity. Stem cell-like features and radioresistance in glioma cells can be promoted by OPN possibly via activation of CD44 signaling through its intracellular domain by enhancing HIF-2α activity (60). Fig. 2 summarizes the mechanisms and signaling pathways activated by OPN on promoting chemoresistance and radioresistance.

4. OPN in the interface of epithelial plasticity and therapeutic resistance

EMT corresponds with the dynamic transdifferentiation of epithelial into mesenchymal cells, in which cells lose their epithelial features and become more motile mesenchymal cells (44). Increasing evidence has demonstrated that there is a close association between EMT and resistance to therapy (59,60), which may be caused by an enhancement of cancer cell survival, cell fate transition, and/or upregulation of drug resistance-related genes during the EMT transition.

The EMT process is mediated by several EMT-inducing TFs (EMT-TF), including TWIST1/2, SNAIL1/2 and zinc finger E-box binding homeobox (ZEB)1/2. In addition to these established EMT-TFs, other TFs have been demonstrated to induce or regulate EMT, including Fox TFs, GATA family, SOX, ovo-like transcriptional repressor 1/2 and grainyhead-like 2 (61,62). Mechanistically, EMT-TFs suppress the expression of key epithelial markers, such as E-cadherin, while activating the expression of mesenchymal genes, such as those coding for vimentin and fibronectin. Collectively, these regulatory networks control the integrity of and the balance between the epithelial and mesenchymal phenotypes. In chemoresistant cells, EMT-TFs increase cell survival in response to therapy-induced programmed cell-death by upregulating anti-apoptotic genes, while downregulating gene products performing pro-apoptotic roles (52). Certain EMT-TFs can also contribute to hormone therapy resistance by modulating the expression of their corresponding receptors (61). These findings make the EMT process an attractive target for reducing chemotherapy resistance (61).

It has also been demonstrated that EMT-TFs act cooperatively with changes at the RNA level that regulate EMT progression, such as alternative splicing and microRNA (miRNA or miR) and long non-coding RNA mediated control of EMT (61-64). EMT process is further controlled by multiple signaling pathways, in which multiple morphogenetic and environmental signals, such as TGF-β,

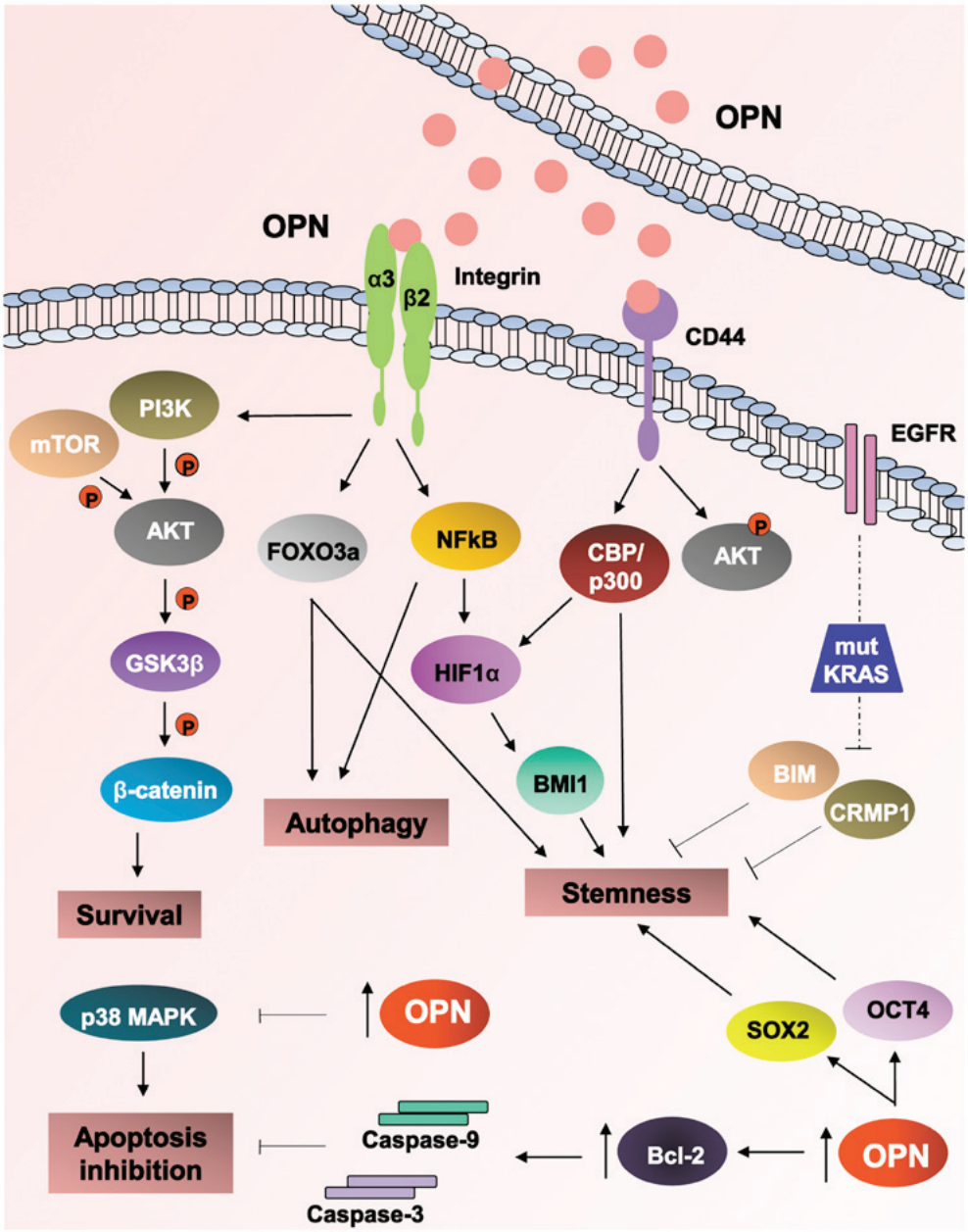

Figure 2. Cancer treatment resistance mechanisms mediated by OPN. Numerous and intricate mechanisms developed by cancer cells have been described at different levels in response to intracellular or extracellular OPN. Upon OPN binding to integrin receptors, especially by αV/β3 heterodimers, the PI3K/AKT/mTOR/β-catenin signaling pathway is triggered, leading to cell survival and chemotherapeutic resistance. OPN can also confer stem cell-like features to cancer cells by sustaining FoxO3a stability-induced autophagy as well as activating the αv/β3/NF-κB/HIF-1α pathway. Accordingly, OPN can modulate cancer stemness by altering CD44 receptor binding to hyaluronate and inducing AKT phosphorylation via CD44, thereby promoting cell survival and chemoresistance. In conjunction with the EGFR pathway, OPN expression, has been associated with radiation resistance particularly in tumors presenting KRAS mut, which was closely associated with the modulation of BIM and CRMP1 expression and thus, stem cell phenotype. Consistently, intracellular OPN was demonstrated to regulate the expression of Oct4 and Sox2, well known modulators of stemness. OPN expression levels have been demonstrated to negatively regulate p38 MAPK and correlate with caspase-3 and -9 blocking and expression of Bcl-2 anti-apoptotic protein, further contributing towards an apoptosis inactivation phenotype. Therefore, OPN has been demonstrated to modulate signaling pathways associated with chemoresistance and radioresistance, through promoting cell survival and inhibiting apoptosis as well as modulating autophagy and stemness in tumor cells. OPN, osteopontin; PI3K, phosphoinositide 3-kinase; AKT, protein kinase B; mTOR, mechanistic target of rapamycin; Fox, Forkhead box; NF-κB; nuclear factor-κB; HIF-1α, hypoxia-inducible factor-1α; Oct4, octamer-binding transcription factor 4; CD, cluster of differentiation; EGFR, epidermal growth factor receptor; mut, mutations; Bcl-2, B cell lymphoma-2; BIM, Bcl-2-like protein 11; CRMP1, collapsin response mediator protein 1; Sox2, sex determining region Y-box 2; MAPK, mitogen-activated protein kinase; GSK3β, glycogen synthase kinase 3β; CBP, cAMP response element binding protein binding protein.

WNT, epidermal and platelet-derived growth factors, inflammatory cytokines and integrin receptor ligands, have been demonstrated to promote EMT in response to extracellular signals (61). Among these molecules, NF-κB and matrix MMPs have also been identified as specific inducers of EMT. NF-κB has been described as a contributor to EMT, by combining with oncogenic gene products, such as RAS in order to protect cells from apoptosis (65). MMPs induce EMT associated with malignant transformation via a pathway dependent upon production of reactive oxygen species (ROS) and degrading matrix proteins, then favoring tumor cell migration and motility (65).

TGF-β, mostly TGF-β1 isoform, is the most well-studied cytokine in the induction of EMT. As reviewed elsewhere (61), TGF-β mediates EMT through mothers against decapentaplegic homolog (SMAD)-dependent or SMAD-independent pathways The SMAD-dependent pathway is initiated by binding of TGF-β1 to TGF-β receptor (TβR)II and TβRI, which then activate SMAD protein complex, which then enter the nucleus to induce the expression of lymphoid enhancer binding factor-1 TF. This TF binds to β-catenin, suppressing transcription of epithelial markers, while promoting the expression of mesenchymal markers. SMAD complexes not only activate the expression, but also increase the activity of EMT-TFs. Other changes in gene expression during EMT occur without directly requiring EMT-TFs, but are rather controlled by TGF-β-activated SMADs, which can directly activate the expression of certain mesenchymal genes. TGF-β also induces signaling through cytoplasmic expression of β-catenin, which is also modulated by the Wnt pathway. In addition to TGF-β-activated SMAD signals, TGF-β also induces EMT signaling through RHO-like GTPases, PI3K/AKT and MAPK pathways. Activation of RHO, RAC and cell division control protein 42 homolog GTPases drives actin reorganization, and lamellipodia and filopodia formation. PI3K signaling activation by TGF-β promotes the activation of mammalian TOR complex (mTORC1 and mTORC2). Notably, mTORC1 and mTORC2 modulate cell size, protein synthesis, motility and invasion. AKT decreases the level of SNAIL1 expression, attenuating E-cadherin repression and the activation of MMP-9 expression. AKT also phosphorylates GSK3β, resulting in SNAIL-1 stabilization. TGF-β also activates ERK, p38 and JUN N-terminal kinase/MAPK pathways, which then also increases TGF-β-induced transcription, leading to increased E-cadherin repression and activation of N-cadherin and MMP expression. EMT signaling has been demonstrated to induce the expression of genes coding for MMP-2 and MMP-9, which cleave Type IV collagen in the basal lamina to promote post-EMT invasion of underlying tissues. MMP-3 has also been demonstrated to directly induce EMT through activation of RAC1 GTPase-reactive oxygen species signaling, which promotes SNAIL1 expression. Additionally, SNAIL1/2 can also promote breakdown of the ECM via upregulation of MMPs (61). Furthermore, MMPs are capable of degrading E-cadherin in the cell membrane. All these processes enable cells to acquire a mesenchymal phenotype (61).

In this context, OPN has previously been reported as a master regulator of epithelial-mesenchymal plasticity, once it has an important regulatory role in the expression of key EMT regulators (66-71). OPN expression also shares functional interplay with the previously mentioned traditional EMT activators, such as TGF-β, TWIST 1/2, ZEB1/2 and SNAIL-family members. Importantly, OPN is able to guide EMT through specific cellular signaling pathways and by restructuring the TME to modify EMT processes (66-71).

OPN overexpression induces TWIST phosphorylation and/or activation through MAPK, AKT and/or RAC/AKT signaling pathways (71,72). Besides, OPN upregulates HIF-1α to induce EMT through TWIST activation and by maintaining the tumor cell stemness (66). TWIST also serves in OPN-mediated metastasis through activation of the PI3K/AKT pathway (66). Signaling mediated by OPN interacts directly or indirectly with ZEB TF family members, as well as leading to EMT. Notably, it is known that OPN is a potent activator of NF-κB (73), which induces the expression of both ZEB1/2 and therefore can regulate NF-κB/ZEB dependent EMT. Likewise, OPN can regulate ZEB-related EMT through non-NF-κB pathways, such as by upregulating the expression of miR-200 family members that inhibit ZEB1/2 initiated EMT (71). Otherwise, NF-κB has also been indicated as a key player of OPN and MMP-9 activation (74). Furthermore, OPN regulates EMT by overexpressing SNAIL EMT-TF. In addition, OPN co-regulates the expression of glioma-associated oncogene, a TF that mediates Sonic hedgehog signaling, which then induces the expression of SNAIL (71). Vimentin upregulation has also been induced by OPN (66). Notably, OPN induces the expression of TGF-β (71).

It has been reported that OPN is able to modify the tissue and TME to support EMT and hence can also indirectly modify EMT (66). During tumor progression, limited tumor oxygen availability and tumor metabolic demands induce hypoxia. As reviewed previously (60,61), the activities of HIF-1α and HIF-2α are enhanced in response to prolonged TGF-β and certain tyrosine kinase receptors. HIF-1α and HIF-2α can then activate EMT-TF expression and/or subcellular localization in tumor cells. Immune and stromal cells from the TME are also sources of EMT-inducing cytokine stimulation. It has been demonstrated that chemotherapy and radiotherapy promote oxidative and inflammatory stress in tumor tissues, contributing to increased expression of inflammatory cytokines and subsequent induction of the EMT program. In the TME, post-translational modifications of EMT-TF can also regulate the half-life of EMT-TFs (61). Malignant signals from the TME with long-lasting effects on associated cancer cells may also modulate epithelial plasticity and then sustain the metastatic potential and tumor chemoresistance. OPN modulates tumor-specific EMT by generating cancer-associated fibroblasts (CAFs), which secrete a multitude of factors in the TME that support tumor invasiveness and metastases, including TGF-β and interleukin-6. Tumor-derived OPN and exogenous OPN are able to induce transformation of CAFs from mesenchymal stem cells by stimulating the production of TGF-β. OPN also enhances the migration and invasion of malignant tumor cells through both the inhibition of apoptosis and by regulating the activities of MMP-2 and MMP-9, which degrade the ECM. OPN has been described to upregulate MMP-9 activity, modulating multiple signaling pathways via focal FAK, ERK and NF-κB that regulate cytoskeletal organization, cell motility, cell growth, and also cell migration, ECM invasion and tumor growth (61,62). In this context, it has been indicated that NF-κB may be a key player in OPN and MMP-9 activation (27). OPN is a substrate for several extracellular proteases and is cleaved *in vitro* and *in vivo* by MMPs. MMP-9, for instance, is known to cleave OPN (75), and it is also known that OPN-regulated signaling leads upregulation of MMP-2 in prostate cancer cells (74). Similarly, OPN can mediate the activation of MMP-9 during migration of prostate cancer and melanoma cells (74).

It has also been reported that OPN has an important role in regulating TGF-β-mediated processes and likely also regulates TGF-β-mediated EMT (70). Considering the emerging roles of OPN in modulating the EMT process, thus contributing to a

drug resistant phenotype, the analysis of OPN expression has been considered as a potential target for future interventions to overcome tumor resistance. Notably, it has been reported that, unlike secreted OPN, which is able to trigger the EMT to initiate cancer metastasis, nuclear OPN is able to induce MET, contributing to metastasis establishment at secondary sites. In this model, OPN interacted with HIF-2α, latterly impacting on the AKT/mir-29/ZEB cascade (71). It has also demonstrated that VEGF in the TME is able to induce OPN nuclear translocation (71).

5. OPN as a target to overcome resistance to cancer therapy

Considering the emerging roles of OPN in several processes associated with chemoresistant and radioresistant phenotype, and its pleiotropic roles in the tumor cascade, the analysis of OPN expression and associated signaling has been considered as a potential target for future interventions trying to overcome tumor resistance, both to early and advanced tumors.

OPN-associated signaling pathways include PI3K/AKT, mTOR, β-catenin or phosphatase and tensin homolog (PTEN) gene expression (72), as well as αvβ3-NF-κB-HIF-1α (49), caspases and Bcl-2 (5,52), p38/MAPK (54) and GSK3β (48), among other targets. Advances in the understanding of the biology of tumor resistance, particularly the signaling pathways associated with this phenotype, may enable the development of novel approaches to overcome resistance to therapy.

Specific and targeted inhibition of these resistance-associated proteins and signaling pathways may potentially increase sensitivity of cancer cells to the cytotoxic action of chemotherapeutic agents and to radiation exposure (44,45). As an example of this approach to further sensitize cells to chemotherapy by targeting OPN and associated signaling, isolated primary $CD34^+/CD38^-$ bone marrow derived acute myeloid leukemia (AML) cells have been treated with curcumin and daunorubicin in combination. This strategy induced AML cell growth inhibition and increased cytotoxicity by upregulating AKT, mTOR, β-catenin or PTEN. Notably, these effects were stronger when OPN expression was specifically knocked-down (72). It has also been reported that OPN/NF-κB-mediated autophagy is required for the maintenance of the stemness state of pancreatic cancer cells, which is associated with survival and chemoresistance (76). These data demonstrated that the blockade of autophagy by downregulating autophagy markers or by treating these pancreatic cells with an autophagy inhibitor reduced the pancreatic CSC populations and associated features, such as the expression of CD44, CD24, CD133 and aldehyde dehydrogenase 1 (76). Once OPN is able to stimulate autophagy and the expression of CSC markers (which includes integrin and CD44 receptors), these cell populations could be prevented by OPN downregulation approaches in order to sensitize pancreatic cancer cells to current chemotherapeutic drugs, such as gemcitabine (76).

Therapeutic application of small interfering RNA molecules targeting OPN (77,78) or neutralizing antibodies associated with OPN epitopes (79) have been tested in order to downregulate OPN expression levels and the results have been promising. An additional approach to sensitize cells to chemotherapy or radiotherapy would be using miRNA molecules targeting OPN or additional gene products associated with chemoresistance, such as those modulating EMT (80,81), drug transporters (78) and cell survival (81). Oncogenic miRNAs are the miRNAs with a defined role in cancer. Several miRNAs are deregulated in cancer cells and correlated with tumor features. Specifically, miRNAs have been reported to influence several tumor-related processes, such as EMT, tumor invasion, metastasis and resistance to therapy (80,82).

Among currently tested miRNAs targeting OPN, a number are able to modulate tumorigenicity, tumor growth and metastasis, such as miR-127-5p (83), hsa-miR-299-5p (80) and miR-181a (84). However, to the best of our knowledge, there is no report describing the specific effects of miRNAs on sensitizing tumor cells to chemotherapy. Conversely, a number of miRNAs that, via targeting OPN, may be promising tools to regulate chemoresistance and radioresistance mediated by OPN. It has been demonstrated that miR-127-5p and hsa-miR-299-5p are able to regulate OPN expression and can respectively modulate human chondrocyte cell proliferation and tumorigenicity, and also display vasculogenic mimicry of spheroid-forming breast cancer cells (80). Similarly, miR-181a regulation of OPN expression provides a novel mechanism of suppressing metastasis in cancer cell lines (84). Furthermore, three lentiviral vectors encoding miRNA against OPN have been reported to inhibit tumor growth and metastasis of human hepatocellular carcinoma, by decreasing MMP-2 and uPA expression, thus leading to inhibition of lung metastasis (85). Furthermore, RNA aptamers have also been proposed to target OPN and it has been demonstrated to decrease EMT and tumor growth (86,87).

Future studies aiming to improve the effects of chemotherapy and radiotherapy could propose similar strategies targeting OPN signaling and additional pathways associated with cancer cell survival and resistance.

6. OPN splice variants and their potential role in tumor resistance

Although previous studies have reported the expression and roles of OPN-SI regarding distinct aspects of tumor progression, data reporting the association between their expression and resistance to therapy are limited. A recent report proposed that OPNb and OPNc splicing isoforms are aberrantly expressed in leukemia cells in response to distinct chemotherapeutic drugs, such as daunorubicin, idarubicin and cytarabine, further mediating resistance to these drugs (88). However, it was not clearly demonstrated that specific knockdown of these splice variants reverted chemoresistance. Our group pioneered the studies of OPN-SI and their relation to chemoresistance, demonstrating that prostate cancer cells that ectopically overexpress OPNb or OPNc are more resistant to docetaxel (DXT) and display higher survival rates (89). The DXT-resistant phenotype was also associated with EMT features in which cells overexpressing OPNb or OPNc exhibited upregulated mesenchymal markers, as opposed to epithelial markers (89). In summary, these data demonstrated that OPN-SI differently modulate chemoresistance. As nuclear OPNc has been demonstrated as a prognostic marker in breast cancer (90) and also reported to be correlated with relapse (90,91) and

poor survival (92), it is possible that nuclear OPNc may also be a potential marker of response to chemotherapy or radiotherapy, as has been previously reported (90). Future studies should further investigate the roles of OPN-SI in chemoresistance in distinct tumor models and also explore their involvement in radioresistance.

7. Conclusions

Growing evidence has pointed to the crucial role that OPN has in many aspects of cancer progression, including the acquisition of drug resistance. OPN not only induces integrin receptor-mediated oncogenic signaling pathways but also modulates the epithelial-mesenchymal phenotype and stemness, conferring cancer cells the ability to survive, proliferate, evade from cell death, migrate and colonize other tissues. Consequently, OPN-overexpressing cells are refractory to current treatment options as well as exhibit invasive and metastatic potential. Together, these findings provide evidence that OPN-triggered signaling pathways can be targeted to specifically induce cell death in chemo- and radio-insensitive cancer cells in order to overcome the therapeutic resistant phenotype. Future studies will uncover the role of specific OPN isoforms in the acquisition of treatment resistance and also address whether OPN and its isoforms may be reliable markers for cancer progression and poor response to standard therapy.

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Authors' contributions

ERPG designed the manuscript, provided financial support, wrote the manuscript, prepared figures, edited and performed major revisions. MCB contributed to figure preparation and revision, as well as writing and editing the manuscript. GNM provided financial support, prepared figures, and contributed to writing and editing the manuscript.

Ethics approval and consent to participate

Not applicable.

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Not applicable.

Competing interests

The authors declare that they have no competing interests.

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