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Review

Osteopontin in Cancer: Mechanisms and Therapeutic Targets

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Abstract: Despite significant advances in the understanding of cancer biology, cancer is still a leading cause of death worldwide. Expression of the tumor microenvironment component, osteopontin, in tumor tissues, plasma, and serum, has been shown to be associated with a poor prognosis and survival rate in various human cancers. Recent studies suggest that osteopontin drives tumor development and aggressiveness using various strategies. In this review, we first provide an overview of how osteopontin promotes tumor progression, such as tumor growth, invasion, angiogenesis, and immune modulation, as well as metastasis and chemoresistance. Next, we address how the functional activities of osteopontin are modulated by the interaction with integrins and CD44 receptors, but also by the post-translational modification, such as proteolytic processing by several proteases, phosphorylation, and glycosylation. Then, we review how osteopontin activates tumor-associated macrophages (TAMs) and cancer-associated fibroblasts (CAFs), and functions as an immunosuppressor by regulating immune surveillance and immune checkpoint in the tumor microenvironment. Finally, we discuss the potential applications of osteopontin as a biomarker and as a therapeutic target.

Keywords: osteopontin; cancer; tumor microenvironment; metastasis; chemoresistance; integrin; CD44; post-translational modification; immune checkpoint; therapeutic target

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1. Introduction

Cancer is a leading cause of death worldwide, accounting for an estimated 19.3 million new cases and almost 10 million deaths in 2020. The global cancer burden is expected to be 28.4 million cases in 2040, a 47% increase from 2020 [1]. Despite significant advances in our understanding of the molecular basis of tumor progression, the development of anticancer drugs remains challenging. However, the discovery of immune checkpoint molecules such as PD-1 led to the development of immune checkpoint inhibitors to control the immune response in cancer [2]. Therefore, a deeper understanding of the molecules that drive tumor progression can enable the development of novel therapeutic strategies for the treatment of this disease.

Tumors are surrounded by complex environmental components called the tumor microenvironment (TME), including the extracellular matrix (ECM), matricellular proteins, vessels, immune cells, fibroblasts, stromal cells, as well as secreted molecules such as hormones, growth factors, and cytokines [3,4]. Recent evidence suggests that the interaction between tumor cells and the TME modulates tumorigenesis, tumor cell invasion, metastasis, chemoresistance, and immune response, which lead to tumor development and aggressiveness. One of the TME components, osteopontin (OPN), was discovered in 1979 by Richard Hynes and colleagues as a transformation-specific phosphoprotein [5]. OPN is a matricellular protein secreted by tumor cells, endothelial cells, fibroblast cells, as well as immune cells within the TME. In recent years, OPN has been increasingly recognized as a critical factor for tumor progression. Many studies have described the overexpression of OPN in tumors and the key roles of OPN in invasion, metastasis, tumorigenesis, chemoresistance, angiogenesis,

and immune suppression [6,7] (Figure 1). Recent studies have shown that the regulation of OPN activities is more complex than originally thought [8–14]. The functional activities of OPN to drive tumor progression are modulated not only by the interaction with receptors but also by the post-translational modification (PTM), such as proteolytic processing by several proteases, phosphorylation, and glycosylation. Furthermore, OPN seems to be a central player in cancer associated with inflammation, which was first proposed by Rudolf Virchow in 1863 [15]. This review will comprehensively summarize recent progress in the field, mainly focusing on the studies within the recent five years that have characterized OPN signaling in the context of tumor progression, post-translational modifications of OPN and its interactions with receptors in cancer progression, and regulation of tumor immunity by OPN. We also discuss recent drug discovery for the OPN-driven cancers and the potential applications of OPN as a biomarker and as a therapeutic target.

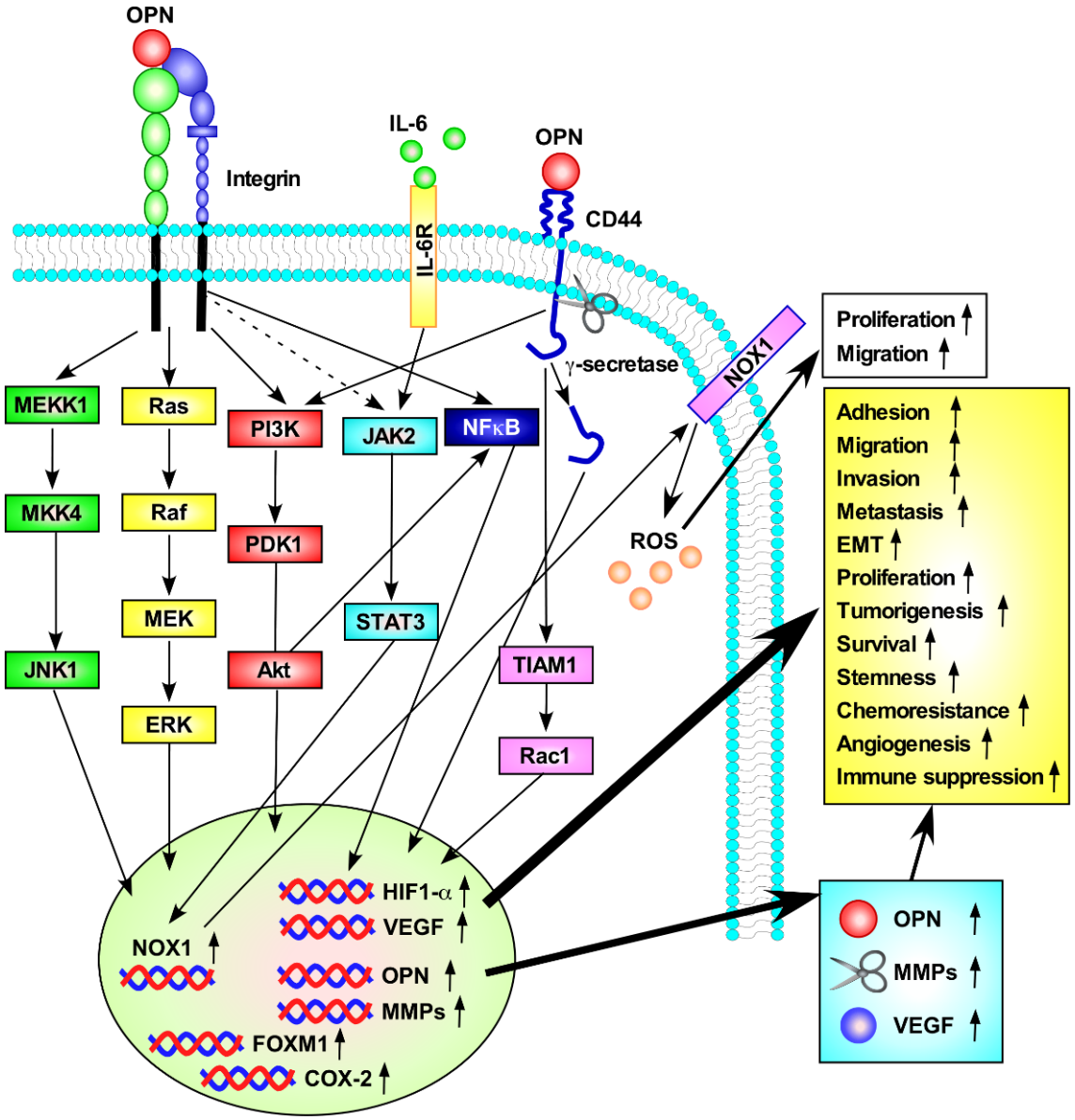

Figure 1. Osteopontin (OPN) signaling in the context of tumor progression in cancer cells. OPN activates JNK, Ras/Raf/MEK/ERK, PI3K/Akt, JAK/STAT, NF-κB, and TIAM1/Rac1 signaling pathways through association with cell surface receptors, integrins, and/or CD44. The OPN-mediated signals induce several gene expressions, such as MMPs and VEGF, and enhance various malignant properties of cancer cells, including invasion, metastasis, tumorigenesis, and chemoresistance.

2. OPN Structure and Functions

OPN (also known as SPP1, ETA-1, BSP-1, and 2ar) belongs to a family of matricellular protein that is a non-structural ECM component [16]. It is found in bone, breast, kidney, lung, nerve, pancreas, and skin tissues [17–19], and is present in body fluids, such as bile, blood, cerebrospinal fluid (CSF), milk, and urine [17,20–22]. OPN is produced in numerous cell types, including epithelial cells, endothelial cells, fibroblasts, pericytes, hepatocytes, lens cells, tubular cells, immune cells (such as T cells, B cells, macrophages, natural killer, natural killer T, and Kupffer cells), neural cells (such as neurons, glial cells, and Schwann cells), osteoblasts, osteoclasts, and vascular smooth muscle cells [23]. The OPNs produced by those cells are involved in various physiological and pathological processes, including wound healing, biomineralization, bone remodeling, vascularization, diabetes, obesity, inflammation, fibrosis, urolithiasis, autoimmune diseases, tumorigenesis, and cancer invasion and metastasis [6,18,20,23–28]. The pleiotropic effect of OPN on these many cellular processes is mainly due to its functional activities, including cell adhesion, migration, proliferation, survival, differentiation, inflammatory cell activation, and immune modulation, which are induced by the association of OPN with cell surface receptors such as integrins and CD44 (Figure 1).

OPN is an intrinsically disordered protein with a highly negative charge (25% of the protein are aspartic or glutamic acid residues) [29] (Figure 2). Intrinsically disordered proteins lack a stable three-dimensional structure, but they have important roles in cell signaling, in protein–protein interactions, and in DNA regulation, and are also associated with several human diseases, such as Alzheimer's disease, Parkinson's disease, and cancer [30,31]. The disorder in intrinsically disordered proteins facilitates their several biological processes, including many PTMs and alternative splicing, which generate complexity and different signaling activities by increasing protein diversity [30,32,33]. Indeed, OPN is subject to various PTMs, including proteolytic processing, phosphorylation, glycosylation, sulfation, and transglutaminase-mediated cross-linking, which allow for a broad range of molecular weights (45 to 75 kDa) and create functional diversity [25].

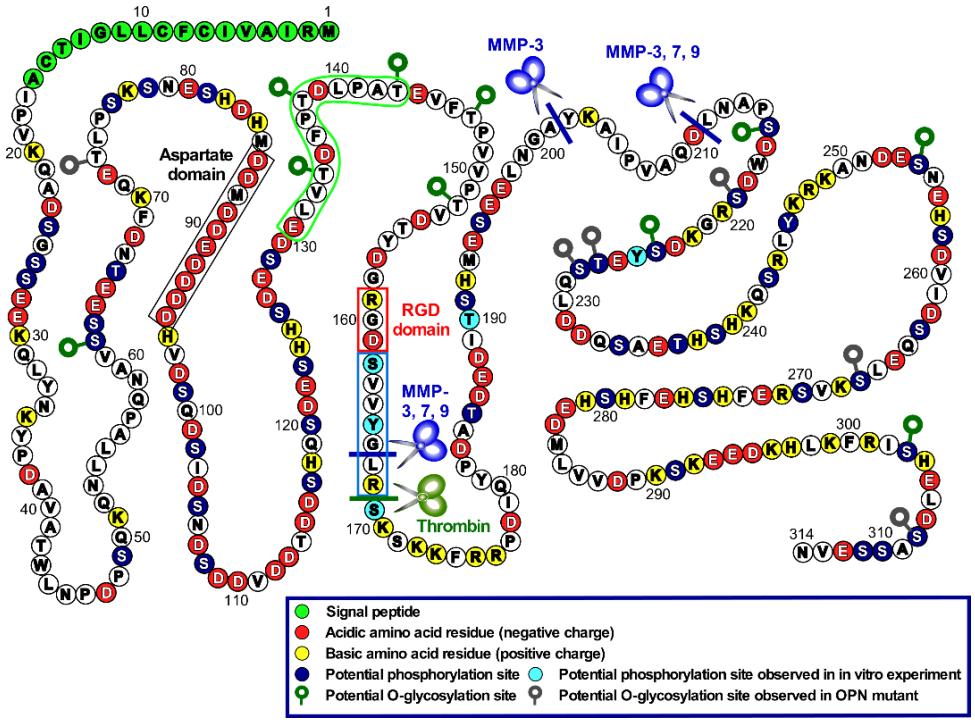

Figure 2. Primary structure and post-translational modifications of osteopontin. Potential phosphorylation and O-glycosylation sites, protease cleavage sites, integrin binding and aspartate domains, and signal peptide are highlighted. Integrin binding domains (E^{131}LVTDFPTDLPAT^{143}, R^{159}GD^{161}, and S^{162}VVYGLR^{168}) and aspartate domain (D^{86}DMDDEDDDD^{95}) are in boxes.

OPN also undergoes alternative splicing. The human OPN gene is composed of 7 exons and OPN has at least 5 alternative splicing variants, OPN-a (all exons present, 314 amino acids), OPN-b (missing exon 5, 300 amino acids), OPN-c (missing exon 4, 287 amino acids), OPN4 (missing exons 4 and 5, 273 amino acids), and OPN5 (containing an extra exon, 327 amino acids) [34]. All OPN variants contain highly conserved sites: matrix metalloproteinase (MMP) and thrombin cleavage sites, potential calcium binding site (D^{216}–S^{228}), two putative heparin binding sites (Y^{165}–F^{174} and K^{296}–I^{302}), and cell surface receptor integrin binding sequences (E^{131}LVTDFPTDLPAT^{143} for α4β1 integrin, R^{159}GD^{161} for α5β1, α8β1, αvβ1, αvβ3, αvβ5, and αvβ6 integrins, S^{162}VVYGLR^{168} for α4β1, α4β7, and α9β1 integrins) (Figure 2). The cell surface receptor CD44 v6 and v7 isoforms bind to both N-terminal and C-terminal regions of OPN, independently of the RGD sequence [35]. Meanwhile, alternative translation of OPN generates both intracellular and secreted OPN isoforms and the two OPN isoforms cause the imbalance of leukocyte populations [36].

3. OPN Expression in Tumors

OPN is highly expressed in many types of tumors, including cutaneous, head and neck, thyroid, breast, lung, esophageal, gastric, liver, pancreatic, colorectal, kidney, bladder, prostate, ovarian cancers, melanoma, myeloma, osteosarcoma, and glioblastoma [6,37–39]. In TME, OPN is primarily expressed in tumor cells, stromal cells, and tumor-infiltrating myeloid cells [40–43]. In clinical studies, OPN expression in tumor tissues, plasma, and serum has been shown to be associated with patient's advanced stage, grade, tumor size, invasiveness, metastasis, and poor survival rate in various human cancers [38,39,44]. Although OPN splicing variants are differentially expressed in different types of cancer, the relationship between the expression of OPN variants and poor outcomes in tumors remains elusive [39,45].

4. Regulation of OPN Expression in Tumors

Expression of OPN in cancer cells is upregulated by various transcription factors, including GLI1, GLI2, Myc, Oct1, Oct4, RUNX1, RUNX2, RUNX3, Sp1, Slug, and TBX3iso1 [46–52]. In hepatocytes, YAP/TEAD4 induces OPN transcription, which stimulates c-Met expression in endothelial cells and then contributes to the formation of a tumor-supporting vascular microenvironment [53]. In contrast, transcription factor IRF8 represses OPN expression by binding to the *SPP1* promoter region in colon epithelial cells, while in colon carcinoma, IRF8 expression is silenced and thereby OPN expression is elevated [54]. In addition, OPN expression is enhanced by ADAM8, mTORC1, NRP2, S100A4, a cholesterol biosynthesis enzyme squalene synthase, TGF-β, as well as mechanical stimuli including matrix stiffness [55–61]. Surprisingly, Chang et al. have reported that chemotherapeutic agent 5-fluorouracil (5-FU)-generated tumor cell debris stimulates OPN expression in both tumor cells and host macrophages, leading to colon carcinoma tumor growth [62]. OPN expression is also regulated by several miRNAs. miR-196a upregulates the expression of OPN by increasing the expression of RUNX2 and thereby promotes hepatocellular carcinoma (HCC) progression [63]. In contrast, the miR-181a/b/c/d are potential miRNAs that target OPN and are downregulated in $CD11b^+$ macrophages from glioblastoma tumors compared to their matched $CD14b^+$ blood monocyte cells. Indeed, overexpression of miR-181a/b/c/d in macrophages and the glioblastoma GL261 cell line leads to decreased OPN production [64]. Other miRNAs such as miR-218-5p, miR-466, and miR-3163 also target OPN and regulate OPN expression [65–67]. Epigenetic regulation can also modulate the transcriptional activity of OPN [68]. Histone methyltransferase WDR5-mediated H3K4me3 methylation increases OPN expression in pancreatic tumor and myeloid-derived suppressor cells [69].

5. OPN in Tumor Progression

OPN plays pivotal roles in tumor development and progression. In lung adenocarcinoma, OPN is functionally involved in early stages of airway epithelial carcinogenesis driven by smoking and mutant $KRAS^{G12C}$ [70]. Binding of OPN and its pro-

teolytic fragments to cancer cells activates various signaling pathways, including JNK, Ras/Raf/MEK/ERK, PI3K/Akt, JAK/STAT, NF-κB, TIAM1/Rac1, and p38MAPK, leading to increased cancer cell adhesion, spreading, migration, invasion, metastasis, epithelial–mesenchymal transition (EMT), proliferation, tumor growth, survival, chemoresistance, stemness, angiogenesis, and immune suppression [8,54,71–79] (Figure 1). Recent studies have revealed novel mechanisms of OPN-mediated tumor progression. Upregulation of OPN expression by parathyroid hormone-related protein (PTHrP), which is frequently amplified as part of the *KRAS* amplification in patients with pancreatic cancer, promotes pancreatic ductal adenocarcinoma cell migration and metastasis [80]. The exosomal S100A4 that is derived from highly metastatic HCC cells activates OPN transcription via STAT3 phosphorylation and thereby promotes the metastatic potential in low metastatic HCC cells. Indeed, HCC patients with both high plasma exosomal S100A4 and plasma OPN levels have a poor prognosis [55]. Moreover, OPN promotes HCC cell proliferation and migration by increasing reactive oxygen species (ROS). This OPN-mediated ROS production is induced by stimulating JAK2/STAT3/NADPH oxidase 1 (NOX1) signaling [71]. OPN also promotes small-cell lung cancer and colorectal cancer cell proliferation by inhibiting autophagy and apoptosis [77,81]. Furthermore, YAP-dependent transcriptional induction of OPN stimulates c-Met expression in continuous endothelial cells. The c-Met expression sensitizes the continuous endothelial cells to the promigratory effects of liver sinusoidal endothelial cell-derived HGF, which contribute to the formation of a tumor-supporting microenvironment in liver tumorigenesis [53].

Cancer cells secrete several types of matrix-degrading proteases, such as MMPs. The proteases degrade the basement membranes consisting of laminins and type IV collagen and the stromal ECM barriers that are mainly composed of type I collagen and fibronectin. The degradation of ECM proteins by the proteases enables cancer cells to invade into the stromal tissues. Since OPN promotes the secretion and activation of MMPs as well as the secretion of urokinase-type plasminogen activator (uPA) in cancer cells, OPN has the potential ability to enhance cancer cell invasion [82,83].

Collectively, these studies indicate that OPN plays key roles at various stage of tumor progression. To understand the mechanisms underlying how OPN promotes tumor progression, this section first discusses OPN receptors and their relationship to the progression of tumors, and next discusses how OPN induces EMT and cancer stem cell (CSC) properties, chemoresistance, tumor angiogenesis, senescence, and bone metastasis, which are key events for tumor progression.

5.1. OPN Receptors and Their Relationship to the Progression of Tumors

5.1.1. Integrin Receptors

The interactions of OPN with cancer cells are mainly mediated through integrin receptors. Integrins are a large family of heterodimeric receptors consisting of α and β subunits and are cell surface adhesion receptors for ECM proteins (e.g., fibronectin, collagen, laminin) and matricellular proteins (e.g., OPN, tenascin, periostin). In mammals, 18α and 8β subunits have been identified, and the combination of them forms 24 distinct integrins. Integrin α and β subunits are both type I transmembrane proteins composed of a large extracellular domain, a single transmembrane domain, and a short (~30–40) cytoplasmic domain (except β4 integrin) [84,85]. Ligand binding to the extracellular domain of integrins or talin binding to the cytoplasmic domain of integrin β subunit triggers a large conformational change from bent closed (inactive) to extended open (active), leading to integrin activation [86]. The active integrins connect to the actin cytoskeleton via talin and kindlin, which induce integrin clustering, activation of focal adhesion kinase (FAK) and Src family kinases, and the initiation of integrin downstream signaling [84,86]. OPN can interact with α5β1, α8β1, αvβ1, αvβ3, αvβ5, and αvβ6 integrins via the $R^{159}GD^{161}$ sequence and with α4β1, α4β7, and α9β1 integrins via $E^{131}LVTDFPTDLPAT^{143}$ and/or $S^{162}VVYGLR^{168}$ sequences [87] (Figure 2). α5β1 and αvβ3 integrins are usually expressed at low or undetectable levels in healthy adult epithelia but are highly upregulated in

cancer, and their expression levels are correlated with disease progression in various tumor types [88]. αvβ5 and αvβ6 integrins are strongly expressed not only in normal mammary and lung epithelium and in renal tubular cells, but also in tumor cells derived from those cells [89]. α4β1, α5β1, αvβ3, and αvβ5 are expressed on blood vessels, and α4β1 and α9β1 integrins are expressed in lymphatic vessels during angiogenesis [90,91]. These integrins promote endothelial cell migration and survival and thereby regulate angiogenesis and lymphangiogenesis. In addition, α4β1 and α4β7 integrins are expressed on leukocytes (lymphocytes, eosinophils, monocytes, macrophages, natural killer cells, basophils, and mast cells), and α9β1 integrin is widely expressed on smooth muscle and epithelial cells, neutrophils, and macrophages [92–95]. These integrins regulate immune cell migration. A quite recent report has shown that αvβ6 integrin in the prostate cancer cell-derived small extracellular vesicles enhances the angiogenic potential of microvascular endothelial cells [96]. In contrast, the role of α8β1, αvβ1, and α4β7 integrins in cancer is so far unknown.

Among the integrins, αvβ3 integrin is the primary receptor for OPN and the OPN/αvβ3 integrin signaling promotes cell adhesion, migration, invasion, proliferation, survival, stemness, angiogenesis, chemoresistance, tumorigenesis, and metastasis [6,72,97–99] (Figure 1). Recent reports have shown that engagement of αvβ3 integrin with OPN also promotes a metabolic shift towards glycolysis [100,101]. Aerobic glycolysis, known as the Warburg effect, is a well-recognized hallmark of tumor cells, and the increased aerobic glycolysis supports cancer cell survival, growth, stemness, drug resistance, invasion, and metastasis, leading to a poor prognosis in cancer patients [102,103]. The OPN/αvβ3 integrin-inducing glycolysis is mediated through NF-κB signaling in HCC cells or through FAK and protein arginine methyltransferase 5 (PRMT5) in glioma cells [100,101]. Intriguingly, in the glycolysis pathway, OPN-a increases the intracellular glucose levels, and OPN-c utilizes this glucose to generate energy, suggesting that OPN/αvβ3 integrin signaling may participate in regulating the Warburg metabolism [104]. Therefore, OPN/αvβ3 integrin signaling plays a pivotal role in cancer progression and this interaction may be a potential therapeutic target for cancer.

5.1.2. CD44 Receptors

Another cell surface receptor for OPN is CD44. CD44 is a type I transmembrane glycoprotein and consists of three domains: an extracellular domain, a transmembrane domain, and an intracellular domain [105]. The CD44 gene undergoes alternative splicing, resulting in the production of standard (CD44s) and variant (CD44v) isoforms. CD44v isoforms may contain a single-variant exon such as CD44v6 and CD44v7, or multiple variant exons such as CD44v4–v5 and CD44v3–v10 [106]. CD44s is ubiquitously expressed on various types of cells, while CD44v isoforms are expressed mainly on epithelial cells and leukocytes [6]. CD44 functions as a cell surface adhesion receptor for hyaluronic acid, collagens, MMPs, as well as OPN [105]. OPN has at least two binding sites for CD44 because each of the two OPN fragments generated by thrombin cleavage can bind to CD44 independently of the RGD sequence [35] (Figure 3a). One of the CD44 interaction sites may be downstream of the RGD motif but overlap with the SVVYGLR domain because OPN–CD44 engagement seems to compete with α9β1 integrin but not αvβ3 integrin [107]. Efficient binding of OPN to CD44 may be required for structural constraint of OPN by immobilization or by binding of heparin (Figure 3a), while the interaction between them is independent of glycosylation [108].

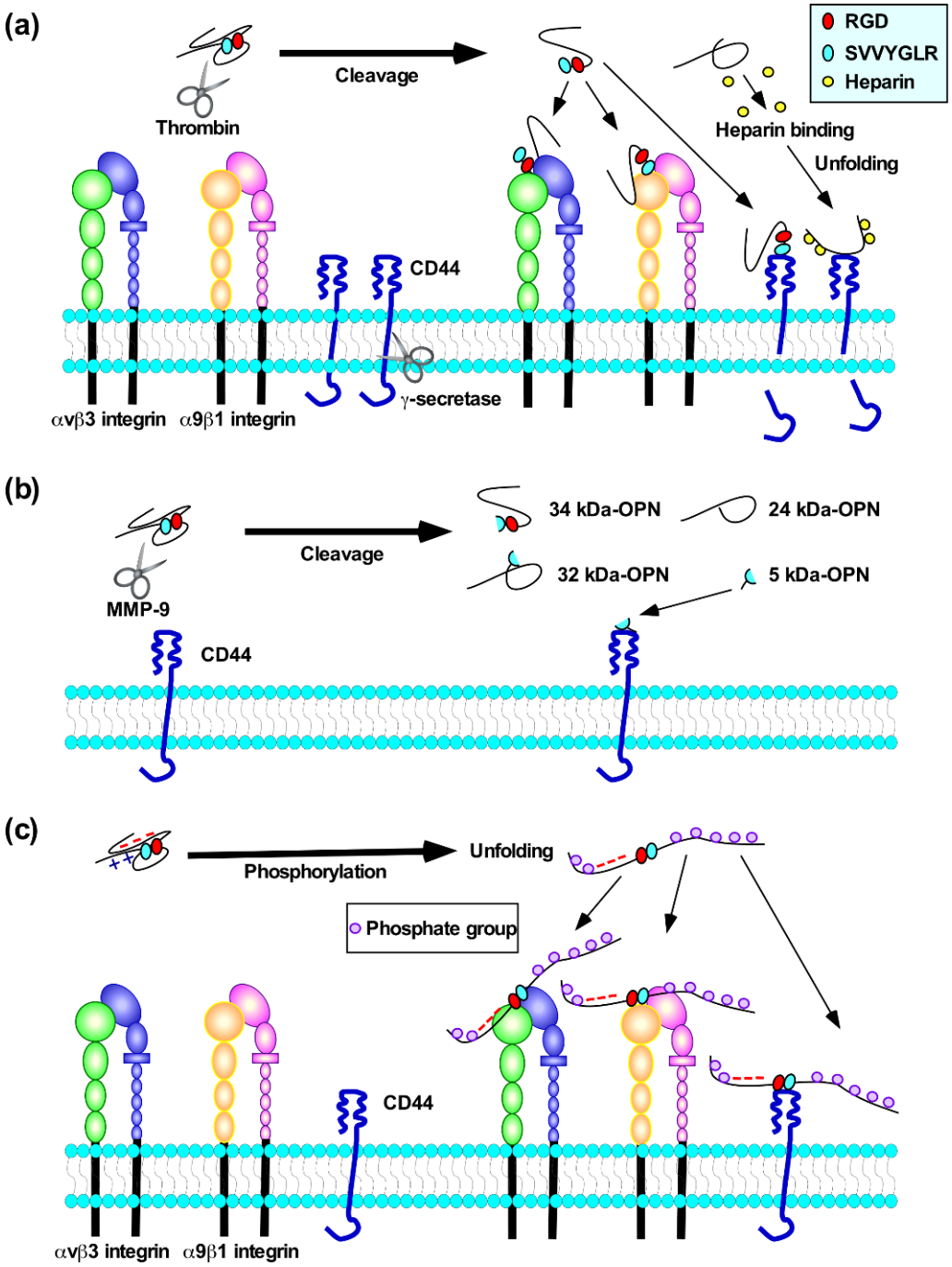

Figure 3. Post-translational modifications of osteopontin (OPN) and its interactions with receptors in cancer progression. (**a**) After cleavage of OPN by thrombin, N-terminal fragment may associate with αvβ3 and α9β1 integrins as well as CD44, while the C-terminal fragment bound to heparin may associate with CD44. (**b**) Cleavage of MMP-9 generates four OPN fragments, and a 5 kDa-OPN fragment may bind to CD44. (**c**) Phosphorylation of OPN may inhibit the internal interaction, probably between positive and negative charge residues, leading to unfolding, and then associate with integrins and/or CD44.

CD44 is extensively expressed in various types of cancers, and is relevant to tumor progression, such as invasion, metastasis, tumorigenesis, stemness, angiogenesis, and chemo/radio-resistance [106]. OPN increases cell surface expression of both CD44s and CD44v in the human melanoma cell line M21 and the prostate cancer cell line PC3 [105]. The OPN–CD44 interaction is known to drive tumor progression. Binding of macrophage-

secreted OPN to CD44s activates Rac-specific guanine nucleotide exchange factor TIAM1 and thereby promotes bladder cancer cell invasion and clonal growth [78]. In addition, the OPN–CD44 interaction promotes tumorigenicity and clonogenicity of colorectal cancer cells through JNK activation [76]. CD44, especially CD44v isoforms, are well-known markers for cancer stem cells in several cancer types and play critical roles in regulating the properties of cancer stem cells, including self-renewal, tumor initiation, metastasis, and chemo/radio-resistance [109]. OPN–CD44 signaling promotes a stemness signature in pancreatic cancer cells and gliomas [110,111]. Mechanistically, OPN induces the cleavage of the intracellular domain of CD44 by γ-secretase, and then the cleaved fragment promotes a stem cell-like phenotype via CBP/p300-dependent enhancement of HIF-2α activity, resulting in aggressive tumor growth in glioma cells [111] (Figures 2 and 3a). Thus, CD44 is involved in transducing the OPN signals for driving cancer progression to cancer cells.

5.2. Role of OPN in Key Events for Tumor Progression

5.2.1. EMT

In many cancers, OPN and αvβ3 integrin induce EMT, which plays a pivotal role in tumor progression [112,113]. EMT is a process whereby cells lose characteristic features of epithelial cells, such as polarity and cell–cell contact, followed by acquisition of the motile mesenchymal phenotype via cytoskeletal reorganization. In cancer, EMT contributes to tumorigenesis, invasion, metastasis, stemness, chemoresistance, and immune evasion. Its reverse process, mesenchymal–epithelial transition (MET), is also important for metastatic colonization and outgrowth [114,115]. In non-small cell lung cancer (NSCLC) tissues, expression of OPN is closely related to EMT, lymph node metastasis before operation, and postoperative recurrence or metastasis [116]. In vitro, OPN-induced EMT promotes lung cancer cell migration, invasion, and proliferation through the activation of the RON tyrosine kinase or PI3K/Akt and MAPK/Erk1/2 signaling pathways [116,117]. Likewise, OPN modulates EMT and cancer stem-like properties in pancreatic cancer cells by activating the αvβ3 integrin-Akt/Erk-FOXM1 cascade [72]. Intriguingly, secretory OPN triggers the EMT to initiate cancer metastasis, while intracellular/nuclear OPN (iOPN) induces the MET to facilitate the formation of metastasis [118]. This result indicates that OPN promotes metastasis by regulating epithelial–mesenchymal plasticity in both early and advanced tumors. Emerging evidence suggests that EMT is not a binary process of these two transition states but instead a broad spectrum of intermediate or partial phenotypes [114,119]. αvβ3 integrin possesses the ability to induce partial EMT, which is characterized by the simultaneous expression of epithelial and mesenchymal markers, and enhances migration, invasion, tumorigenesis, stemness, and metastasis [120]. Thus, OPN/αvβ3 integrin signaling may promote tumor progression by regulating the EMT, MET, as well as partial EMT.

5.2.2. CSC Property

CSCs are self-renewing multipotent cells, which are also suggested to be responsible for chemotherapy resistance. OPN promotes a CSC-like phenotype and chemoresistance via activating NF-κB/HIF-1α and PI3K/Akt signaling pathways in HCC, colorectal cancer, and glioma cells [75,121–123]. Cancer-associated mesothelial cell-secreted paracrine OPN, which is induced by TGF-β produced in ovarian cancer cells, also promotes ovarian cancer chemoresistance and stemness [74]. Likewise, paclitaxel-induced OPN promotes stem cell properties and chemo-resistant metastasis via JNK signaling in breast cancer cells [124]. Yang et al. have reported that the induction of autophagy by OPN/NF-κB signaling is required for maintenance of pancreatic CSC properties and resistance to gemcitabine [125]. The OPN-induced autophagy promotes chemoresistance via binding with αvβ3 integrin and sustaining FoxO3a stability in HCC [126]. Several cell surface markers, such as CD133, CD44, CD24, EpCAM, and aldehyde dehydrogenase 1 (ALDH1), have been identified as pancreatic CSC markers. Indeed, high OPN/CD44/CD133 co-expression and high OPN/autophagy marker LC3/ALDH1 co-expression are associated with poor overall survival and disease-free survival in patients with pancreatic cancer [125].

Epidermal growth factor receptor-tyrosine kinase inhibitors (EGFR-TKIs), including gefitinib and afatinib, are effective for NSCLC with activating mutations in EGFR (e.g., deletions in exon 19 and the exon 21 L858R mutation) [127]. However, most patients treated with EGFR-TKIs eventually develop acquired resistance to them. The most common mechanism that underlies the resistance is the T790M mutation, which accounts for approximately 55% of acquired resistance to the EGFR-TKIs [127]. In EGFR-TKI-resistant NSCLC cell lines with an EGFR mutation, PC9 (EGFR mutation: deletions in exon 19 and T790M), H1650 (EGFR mutation: deletion in E746-A750), and H1975 (EGFR mutation: T790M, L858R), OPN expression is apparently higher than that in parental cell lines [127–129]. The secreted OPN contributes to acquire EGFR-TKI resistance by activating the αvβ3 integrin-FAK/Akt and ERK signaling pathways as well as EMT induction in NSCLC cell lines [127,128]. Therefore, OPN inhibitors may be a better therapeutic option for NSCLC patients who develop the acquired resistance to EGFR-TKI. Knowledge about the molecular mechanisms underlying the acquired resistance will be helpful for better understanding and overcoming the acquired resistance to EGFR-TKI in NSCLC [127,128].

5.2.3. Chemoresistance

Resistance to chemotherapy is a major cause of mortality in advanced cancer [130]. OPN expression is associated with drug resistance in several types of cancers [34]. The cell lines derived from a fast-growing mouse breast tumor are very insensitive to apoptotic stimuli and selectively overexpress OPN [131]. Similarly, chemotherapeutic agents such as paclitaxel, doxorubicin, 5-FU, and methotrexate induce OPN expression in breast cancer cells through activation of JNK signaling [124]. The breast cancer cell lines treated with doxorubicin prevent caspase-3-induced apoptosis through OPN-mediated activation of MAPK/MEK1/2 signaling pathways [131]. OPN also contributes to acquire drug resistance in cancer cells by increasing the expression of drug efflux transporter [130]. OPN/αvβ3 integrin engagement increases P-glycoprotein expression [99], which is a multi-drug efflux transporter and is responsible for drug resistance in cancer cells. In prostate cancer cell line PC-3, knockdown of OPN enhances the cell death caused by daunomycin, paclitaxel, doxorubicin, actinomycin-D, and rapamycin, as well as suppresses tumorigenesis by treatment with daunomycin in a mouse model [99]. The OPN secreted from cancer-associated mesothelial cells facilitates ovarian cancer cell chemoresistance via the activation of CD44-mediated PI3K/Akt signaling and ABC drug efflux transporter [74]. Thus, OPN may induce drug resistance by preventing apoptosis signals and upregulating the expression of drug efflux transporter.

5.2.4. Tumor Angiogenesis

Angiogenesis, the formation of new blood vessels from preexisting vessels, is required for tumors to acquire oxygen and nutrients essential for their growth and metastasis [90]. The value of OPN as an angiogenic factor has already been confirmed by several studies. When comparing the serum concentration of six angiogenesis markers, OPN is the best single angiogenesis marker in blood samples collected from patients with ovarian cancer [132]. Upregulation of OPN by a transcriptional regulator, TBX3iso1, in breast cancer cells promotes angiogenesis in an in vivo mouse model and in an in vitro tubule formation assay using human dermal microvascular endothelial cells [48]. Tumor cells secrete vascular endothelial growth factor (VEGF) as an angiogenic factor to promote angiogenesis and OPN is likely to contribute to the VEGF production. OPN augments the expression of VEGF in breast cancer cells via the Brk/NF-κB/activating transcription factor-4 (ATF-4) signaling pathway and in endothelial cells via PI3K/Akt and αvβ3 integrin/ERK1/2 signaling pathways, leading to tumor angiogenesis [79,133,134]. Hypoxia-driven OPN induces integrin-linked kinase (ILK)/Akt-mediated NF-κB activation, leading to HIF-1α-dependent VEGF expression in breast cancer cells and the following angiogenesis in response to hypoxia [135]. Thus, OPN most likely promotes angiogenesis by upregulation of VEGF in both cancer and endothelial cells.

In the TME, OPN promotes angiogenesis in collaboration with macrophages. OPN activates the PKCα/c-Src/IκB signaling pathway in prostate cancer cells as well as ERK and p38 signaling via α9β1 integrin in macrophages, leading to cyclooxygenase-2 (COX2)/prostaglandin E2 (PGE2)-stimulated angiogenesis [95,136]. A disintegrin and metalloproteinase (ADAM)8 is a proteolytically active member of the ADAM family. In breast, gastric, colorectal, liver, and pancreatic cancers, and glioma, the high expression levels of ADAM8 are involved in tumor cell migration, invasion, and tumorigenesis, and correlated with a poor patient prognosis [56,137]. ADAM8 upregulates OPN expression via the JAK/STAT3 pathway in GBM cells and macrophages, thereby promoting angiogenesis [56]. Furthermore, a pro-inflammatory cytokine IL-18 acts synergistically with IL-10 to amplify the production of OPN and thrombin in macrophages, yielding the generation of a thrombin-cleaved form of OPN [138]. Subsequently, the thrombin-cleaved OPN binds to α4/α9 integrins on macrophages, which in turn augment M2 polarization of macrophages with higher expression of CD163. The CD163 may be responsible for mediating the direct interactions between macrophages and endothelial cells, ultimately resulting in the excessive angiogenesis [138]. Thus, OPN may influence angiogenesis by activating pro-angiogenic signaling in both cancer cells and macrophages.

5.2.5. Senescence

Senescent cells survive and accumulate in the body, and secrete a variety of secreted proteins, cytokines, chemokines, growth factors, and proteases, termed the senescence-associated secretory phenotype (SASP). SASP is now considered to be associated with tumor progression, and OPN is known as a SASP factor [139,140]. Stewart et al. have reported that stromal cell-derived OPN, which is regulated by c-Myb and C/EBPβ, contributes to preneoplastic cell growth through activation of the MAPK pathway [73,141]. Additionally, senescent fibroblasts in the TME facilitate invasiveness and metastasis of breast cancer cells through degradation of the Rac exchange factor Tiam1 and the consequent increase in secretion of OPN by fibroblasts [142,143]. Furthermore, the immediate early-response gene IER2 expression correlates with poor prognosis in melanoma patients and induces senescence in melanoma cells in a p53/MAPK/AKT-dependent manner. The IER2-mediated senescent melanoma cells produce SASP factors including high levels of OPN, and the secreted OPN strongly stimulates the migration and invasion of non-senescent melanoma cells [144].

5.2.6. Bone Metastasis

Bone metastasis is most common in patients with breast, prostate, or lung cancers, and is often painful and reduces the survival of patients. OPN is known as a bone metastasis-related protein [7,145]. Conditional knockdown of OPN inhibits skeletal metastasis of breast cancer MDA-MB231 [146]. Additionally, overexpression of αvβ3 integrin in the MDA-MB231 cell line increases bone metastasis incidence and promotes both skeletal tumor burden and bone destruction in mouse models [147]. In fact, increased serum levels of OPN are observed in NSCLC patients with bone metastasis [148]. PTHrP is frequently overexpressed in patients with bone metastasis in lung, breast, head and neck, lymphoma, pancreatic, and colon cancers, and the increased PTHrP expression levels correlate with reduced survival [80,149,150]. Since PTHrP can drive OPN expression through Runx1 and Runx2 upregulation [49,50,149], OPN may play a pivotal role in bone metastasis in concert with PTHrP.

6. PTM of OPN in Tumors

The majority of extracellular proteins undergo PTMs that are enzyme-mediated biochemical modifications after protein biosynthesis. These modifications increase functional properties of proteins and thereby regulate molecular and cellular activities, such as cell adhesion, migration, proliferation, and survival [151,152]. PTMs preferentially occur in intrinsically disordered proteins because of their structural pliability in potential modification sites that is required for the efficient association with modifying enzymes [153]. Recent

studies have shown that the functional activities of OPN are modulated by the PTM in the TME (Figure 3). This section focuses on the role of three major PTMs on OPN, proteolytic processing, phosphorylation, and glycosylation, in tumor progression.

6.1. Proteolytic Processing

Proteolytic processing alters the OPN structure and functions, which in turn drive tumor progression. OPN undergoes proteolytic processing by thrombin and MMP-2, -3, -7, and -9, which are derived from both the host and tumor cells [154–157]. OPN has a highly conserved thrombin cleavage site at R^{168}–S^{169} near the integrin binding $R^{159}GD^{161}$ domain. OPN also undergoes proteolytic cleavage at G^{166}–L^{167}, A^{201}–Y^{202}, and D^{210}–L^{211} by MMP-3 and at G^{166}–L^{167} and D^{210}–L^{211} by MMP-7 and MMP-9 (Figure 2). The fragments generated by proteolytic cleavage have different functions from each other and full-length OPN. Thrombin-cleaved OPN fragments are markedly increased in both CSF and tissue samples from glioblastoma patients [156]. The cleaved OPN promotes cell migration and confers resistance to apoptosis in glioblastoma cell line T98G [156]. Recent work from Leung's lab has reported that suppression of B16 melanoma tumor growth and metastasis is observed in thrombin cleavage-resistant OPN knock-in mice [10]. Similarly, thrombin-cleaved OPN C-terminal fragment, which does not contain the RGD domain, increases RGD-independent cancer cell migration and invasion via CD44 variant/β1 integrin or cyclophilin C/CD147 [35,158]. MMP-9 cleaves OPN at two predominant sites (residues 166 and 210), generating four fragments, that is, 34 kDa-OPN (residues 1–166), 32 kDa-OPN (residues 167–314), 24 kDa-OPN (residue 211–314), and 5 kDa-OPN (residues 167–210). The 5 kDa-OPN fragment promotes HCC cellular invasion via CD44 [157]. These effects of the proteolytic processing on OPN functions are largely due to the alteration of association with its cell surface receptors. Thrombin cleavage of OPN reveals a cryptic binding site for α9β1 integrin (S^{162}VVYGLR^{168}) and the RGD domain, allowing for the α9β1 integrin- and RGD-binding integrin-mediated cell adhesion to the N-terminal fragment of OPN [159,160]. In contrast, the cleavage of recombinant OPN by MMP-3 abolishes the binding of α5β1 and α9β1 but not αvβ5 and αvβ6 integrins [161]. Therefore, the proteolytic processing may modulate the OPN functions in tumor progression. Since OPN can stimulate MMP expression and activity, upregulation of OPN in tumor tissues may induce cancer cell invasion and metastasis by facilitating ECM degradation as well as the OPN processing [82,162]. Another crucial role of proteolytic processing of OPN in tumor progression is suppression of the host anti-tumor immune response. Thrombin-cleaved fragments of host OPN suppress the host anti-tumor immune response by functionally modulating the tumor-associated macrophages [10]. In addition, MMP-9-cleaved fragments induce expansion of myeloid-derived suppressor cells, which contribute to immune evasion of tumor cells [14]. Therefore, OPN-processing enzymes may represent a potential therapeutic target for the treatment of cancers.

6.2. Phosphorylation

Protein phosphorylation is a reversible PTM. It is a major mechanism for regulating the function of proteins through conformational changes and modulation of binding events due to the addition of negatively charged phosphate groups to protein [163]. OPN is an extracellular phosphoprotein with the largest proportion of potential phosphorylation sites (more than 15%) among extracellular proteins [164,165]. In human OPN, 49 potential phosphorylation sites have been identified until now [8,97,164,165] (Figure 2), and the phosphorylation can be influenced by O-glycosylation [8,97]. Mateos et al. prepared a phosphorylated OPN by in vitro phosphorylation reaction using FAM20C kinases and examined the effect of phosphorylation on OPN structure [164]. As a result, the phosphorylation caused a significant structural elongation of OPN, accompanied by an increase in local flexibility, especially in the phosphorylation site-rich C-terminal region (residues 200–314) [164]. Since the N-terminal region (especially around the aspartate domain) highly contains negatively charged amino acids and the C-terminal region highly contains positively charged

amino acids (see Figure 2), intramolecular interactions between N-terminal and C-terminal regions may occur in less phosphorylation states [166]. Once OPN is fully phosphorylated, the phosphorylation in the C-terminal region of OPN may hamper intramolecular interactions between N-terminal and C-terminal regions by the addition of negatively charged phosphate groups. Thus, the structural elongation of OPN by phosphorylation may reveal the cryptic binding sites of integrins, and CD44 [29]. Further studies are required to test this hypothesis.

Previous studies using mouse Ras-transformed fibroblasts, mouse fibroblasts, and mouse osteoblasts have shown that phosphorylation of OPN affects cell adhesion to OPN [25]. Additionally, the experiments using a recombinant mouse OPN purified from *E. coli*., which has no glycan, have shown that phosphorylation is required for functional interaction with integrin but not CD44 [167]. In addition, phosphorylation of the recombinant OPN induces macrophage chemotaxis, spreading, and MMP-9 secretion [167]. Phosphorylation of Ser^{162} at the RGDSVVYGLR motif in recombinant OPN, which is purified from *E. coli*., diminishes cell adhesion via $\alpha v\beta 3$ integrin [13]. Although phosphorylation of OPN regulates its activities, the phosphorylation state of OPN is likely to depend on the species and cell types [25]: does phosphorylation of cancer cell-derived OPN affect its activities? Our recent study has shown that the highly phosphorylated OPN is found in the cell culture media in human lung cancer cell lines A549 and H460, but not in those of human melanoma cell line MDA-MB435S, although those three cell lines predominantly express OPN [9]. The A549 and H460 cell culture media, as well as the MDA-MB435S cell culture media with a kinase treatment, clearly show enhanced cancer cell migration, both of which are abolished by alkaline phosphatase treatment or anti-OPN antibodies. Therefore, phosphorylation of OPN produced by cancer cells may be associated with cancer progression.

The analysis using the Clinical Proteomic Tumor Analysis Consortium dataset revealed that S^{234} of OPN shows higher phosphorylation levels in breast-invasive carcinoma, colon adenocarcinoma, lung adenocarcinoma, and uterine corpus endometrial carcinoma compared with those in normal tissues [37]. Phosphorylation levels are also increased at S^{195}, S^{219}, S^{258}, and S^{280} in breast-invasive carcinoma, S^{62} or S^{63}, S^{219}, S^{254}, and S^{258} or S^{263} in colon adenocarcinoma, S^{62} or S^{63}, S^{258}, and T^{190} in lung adenocarcinoma, and S^{195}, S^{219}, S^{254}, and S^{263} in uterine corpus endometrial carcinoma [37]. Furthermore, phosphorylation levels of OPN at S^{219} in the extracellular vesicles isolated from the urine samples of patients with prostate cancer are significantly higher than those in controls [168]. Tagliabracci et al. have reported that OPN is a substrate for FAM20C, which is a Golgi casein kinase that phosphorylates secreted proteins [169]. Compared with the normal tissues, FAM20C expression is elevated in brain and central nervous system, breast, cervical, esophageal, head and neck, lymphoma, and pancreatic tumors [170]. The high expression of FAM20C is positively associated with the poor prognosis of patients with bladder urothelial carcinoma, brain lower-grade glioma, and stomach adenocarcinoma [170]. The cancer-specific phosphorylation sites identified in clinical samples are FAM20C-dependet phosphorylation sites, except S^{62} and S^{219} [37,168,169], so that phosphorylation of OPN is probably associated with cancer progression via FAM20C. However, the role of phosphorylation at the cancer-specific phosphorylation sites on OPN in cancer progression remains to be clarified by further studies.

6.3. Glycosylation

Glycosylation is the most common PTM of protein, and secreted or membrane-associated proteins are nearly all glycosylated [171]. Indeed, matricellular and ECM proteins, as well as integrins, are well-known substrates for glycosylation [28,172]. Protein glycosylation participates in receptor activation, cell–cell and cell–ECM interactions, inflammation, immune surveillance, cellular signaling, and cellular metabolism [152]. Cellular transformation is accompanied by alteration of the carbohydrate structure on glycoprotein, and the changes in glycans on matricellular and ECM proteins as well as integrins often

lead to increased cancer cell proliferation, migration, invasion, and survival, which are critical for tumor development and progression [12,152,173].

Glycoprotein can covalently attach one or more glycans to a polypeptide backbone, mainly via N-linkage to Asn in the Asn-X-Ser/Thr motif (X is any amino acid except Pro) and via O-linkage to Ser or Thr, and they are termed N-glycans and O-glycans, respectively [151]. One of the O-glycans, mucin-type O-glyans that are initiated by N-acetylgalactosamine (GalNAc) O-linked to Ser/Thr, are frequently found in secreted or membrane-associated glycoproteins [151]. Human OPN contains several mucin-type O-glycans [8,97]. In contrast, there is only one report about N-glycans on human OPN, which is isolated from bone [174], although human OPN has two Asn-X-Ser/Thr motif sequences and the N-glycans on OPN in other species are frequently observed.

Sialic acid is a terminal component of the oligosaccharide chains of many glycoproteins, and the sialic acid on OPN is important for the association of OPN with integrins [175]. Recent studies using O-glycosylation site-defective mutants have shown that O-glycans on OPN play important roles in cancer cell adhesion, migration, proliferation, and association with αvβ3 and β1 integrins, as well as tumor growth in mouse models [8,11,97]. Furthermore, O-glycosylation of OPN affects MMP-9 expression in cancer cells and phosphorylation of OPN [8,11,97]. The lack of O-glycans at Thr^{143}, Thr^{147}, and Thr^{152}, which are proximal to the RGD sequence for binding of αvβ3, α5β1, and αvβ1 integrins, increases the adhesion of OPN to human breast cancer cell line MDA-MB231 and fibrosarcoma cell line HT1080, and also acquires resistance to a function-blocking antibody against αvβ3 and β1 integrins compared to wildtype OPN [97]. Therefore, these effects of O-glycosylation on the OPN functional activities may be caused by alteration of the association of OPN with integrins.

OPN is a carrier of STn antigen [8], which is one of the tumor-associated O-glycans, and is associated with poor prognosis and metastasis in several human cancers [151]. Although the effect of STn addition to OPN on its activities remains unclear, the understanding of the relationship between them might be useful for further advanced comprehension of the role of OPN glycosylation in cancer.

7. OPN and the Immune System in Cancer

OPN was initially identified as an immune regulatory molecule of T cell activation and called early T cell-activated gene (Eta-1) [176]. Under physiological and pathological conditions, OPN regulates the host immune response against infection and immune cell-mediated inflammatory and autoimmune diseases by modulating inflammatory cell adhesion, migration, and activation, as well as T cell differentiation [6]. Additionally, recent studies have revealed that OPN is primarily expressed in tumor cells and tumor-infiltrating myeloid cells in human cancer patients, and plays key roles in tumor immune evasion in the TME [6] (Figure 4). Myeloid regulatory cell- (MRC) and colon carcinoma cell-derived OPNs suppress activation of cytotoxic T lymphocytes (CTLs) via association with CD44 on CTLs [54]. In addition, OPN inhibits the lytic activity of tumor-specific CTLs, leading to the promotion of colon tumor growth [177]. Furthermore, host-derived OPN promotes macrophage recruitment and M2 phenotype polarization, which exhibit immunosuppressive and tumor-promoting functions [138,178,179]. Tumor-derived OPN promotes M2 polarization and myeloid-derived suppressor cell expansion through STAT3 activation and suppresses antitumor immunity by promoting extramedullary myelopoiesis [180–182]. Thus, OPN may promote tumor progression by suppressing the immune system. This section focuses on the role of OPN in tumor-associated macrophage (TAM) activation and the immune checkpoint in the immune system in cancer.

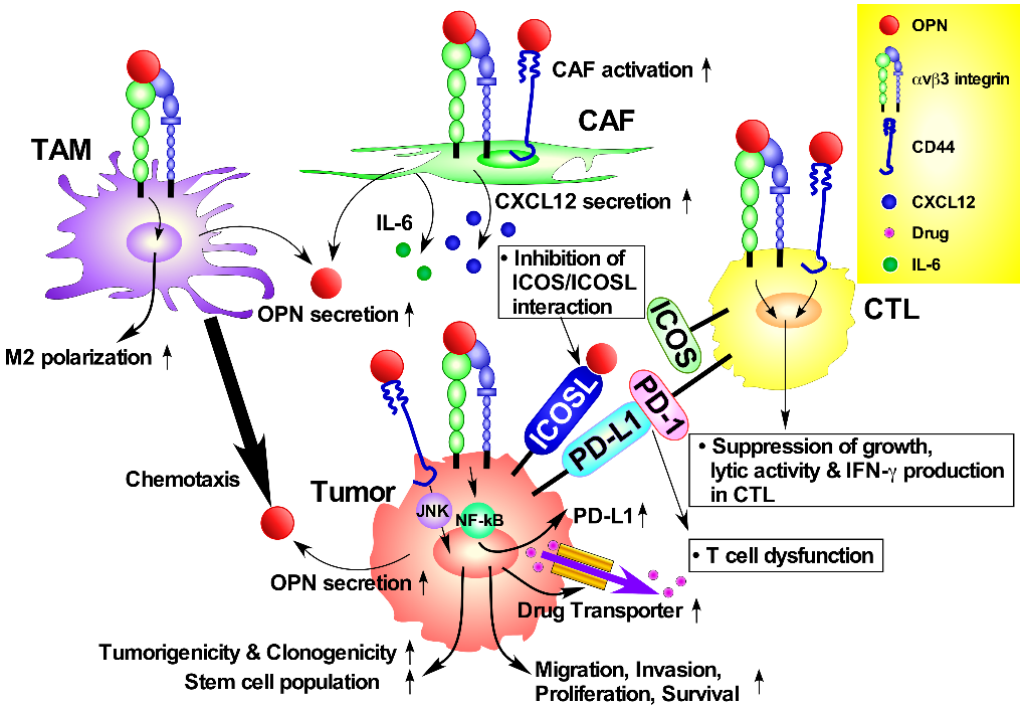

Figure 4. Regulation of tumor immunity by osteopontin (OPN) in the tumor microenvironment. In tumor cells, OPN promotes migration, invasion, proliferation, survival, tumorigenicity, clonogenicity, stem cell population, drug transporter expression, as well as OPN secretion through the integrins and CD44-mediated signaling pathways. In tumor-associated macrophages (TAMs), OPN–αvβ3 integrin engagement induces M2 polarization and OPN production. In cancer-associated fibroblasts (CAFs), OPN promotes IL-6, CXCL12, and OPN secretion through the association with αvβ3 integrin and CD44. OPN inhibits the cytotoxic T lymphocyte (CTL) response through immune checkpoint engagement via PD-L1 expression, binding to ICOSL, as well as suppression of cell proliferation, lytic activity, and IFN-γ production through αvβ3 integrin and CD44-mediated signals in CTL.

7.1. Tumor-Associated Macrophages (TAMs)

TAMs are macrophages that populate in the surrounding TME and are generally associated with poor prognosis and drug resistance in solid tumors [183,184]. TAMs can be classified into two types, proinflammatory "M1" (classical activated macrophages) and anti-inflammatory "M2" (alternative-activated macrophages) phenotypes. M1 macrophages are involved in inflammation responsible for the Th1 cell response to tumor cells and thereby show anti-tumor effects, whereas M2 macrophages promote immune suppression by secreting anti-inflammatory cytokines, leading to tumor progression [183,184].

OPN expression in TAMs is associated with tumor progression. Indeed, patients with a subunit of complement component C1q, $C1QC^{low}$, and OPN^{high} TAMs gene signatures have the worst prognosis, highest proportion (71.79%) of locally advanced cervical cancer, and lowest immune cell infiltration [185]. Likewise, OPN-positive macrophages are associated with tumor progression and worse patient survival in colorectal cancer [186]. In OPN-knockout mice models of melanoma, infiltration of TAMs into tumor tissues and the following melanoma growth and angiogenesis via α9β1 integrin are suppressed [95]. Similar results are observed in OPN-knockout mice models of glioblastoma. OPN deficiency in host cells reduces TAM infiltration and enhances T cell effector activity in infiltrating the glioma [178]. In addition, co-injection or co-culture with patient-derived CD44-positive colorectal cancer cells produce higher levels of OPN production in TAMs but not peritoneal macrophages, which in turn facilitates the tumorigenicity and clonogenicity of colorectal cancer cells through the OPN/CD44-mediated JNK activation [76]. Furthermore, TAM-derived OPN also stimulates cancer cell migration, invasion, proliferation, survival,

angiogenesis, and suppression of the CTL response [187]. These studies indicate that both tumor-derived OPN and TAM-derived OPN are critical for tumor progression (Figure 4).

7.2. Immune Checkpoint

Immune checkpoint molecules are inhibitory receptors expressed on immune cells that suppress immune activation when binding to the specific ligands [188]. Tumor cells hijack the immune checkpoint system to promote an immune-suppressive state that facilitates immune surveillance evasion and tumor growth. For example, the interaction of programmed cell death ligand 1 (PD-L1) on tumor cells with programmed cell death protein 1 (PD-1) on T cells induces T cell dysfunction and allows cancer cells to evade immune surveillance (Figure 4). OPN may act as an immune checkpoint to negatively regulate T cell activation. TAM-derived OPN is able to suppress the anti-tumor immune response by upregulating PD-L1 surface expression in NSCLC cells through NF-κB signaling [189] and in HCC cells via activation of the colony-stimulating factor-1 (CSF-1)/CSF-1R pathway [179]. Therefore, patients with high OPN expression in TAMs may show a poor response to anti-PD-L1 treatment [186].

Another immune checkpoint molecule relating to OPN is inducible T cell co-stimulator (ICOS, also known as CD278), a cell surface receptor mainly expressing on activated T cells [2]. The binding partner of ICOS is ICOS ligand (ICOSL, also known as B7-H2, and CD275), which is a transmembrane protein expressing in B cells, macrophages, dendritic cells, endothelial cells, mesenchymal cells, epithelial cells, fibroblasts, as well as in many primary tumors and tumor cell lines [2]. The interaction of ICOSL with ICOS transduces anti-tumor signals to the cells expressing ICOSL. Recently, Raineri et al. have reported that OPN binds to ICOSL at a different site than ICOS, which promotes cancer cell migration in vitro, and tumor metastasis and angiogenesis in vivo [190,191]. These results suggest that the OPN–ICOSL interaction may suppress the binding of ICOS to ICOSL, thereby blocking anti-tumor signals.

8. OPN and Cancer-Associated Fibroblasts (CAFs)

Cancer-associated fibroblasts (CAFs) are the most abundant and highly heterogenous stromal cells in the TME [192]. CAFs modulate tumor growth, cancer invasion and metastasis, angiogenesis, immune response, and therapeutic resistance through synthesis and remodeling of ECM as well as production of soluble secreted factors, such as growth factors, cytokines, chemokines, and other regulatory factors [192,193]. Recent reports have shown that CAFs can originate from a variety of cells, such as resident fibroblasts, mesenchymal stem cells, and stellate cells [192].

OPN is involved in CAF activation [194] (Figure 4). Breast cancer cell-derived OPN promotes the activation of resident fibroblasts into CAF by the association with CD44 and αvβ3 integrin on the fibroblast cell surface, which mediate signaling through Akt and ERK to induce Twist1-dependent gene expression [195,196]. The OPN-driven CAFs then secrete CXCL12, which in turn induces cancer cell migration, EMT marker expression, angiogenesis, and tumor growth [195]. Furthermore, tumor-derived OPN also transforms mesenchymal stem cells into CAFs through the transcription factor, myeloid zinc finger 1 (MZF-1)-dependent TGF-β1 production, leading to promote tumor growth and metastasis [197]. Similarly, OPN–αvβ3 integrin engagement is able to generate endothelial-derived mesenchymal cells via endothelial–mesenchymal transition (EndoMT) through PI3K/Akt and mTORC1-dependent HIF-1α expression. Like CAFs, EndoMT-derived cells promote tumor growth, invasion, and stemness in colorectal cancer cells by secreting HSP90α [98].

CAF-derived OPN participates in tumor progression [143,198]. In luminal breast cancer, α-smooth muscle actin-positive CAFs are associated with a poor prognosis and a more aggressive phenotype of tumor cell lines in patients with the cancer [43]. The α-smooth muscle actin-positive CAFs isolated from luminal breast tumors overexpress OPN and the OPN expression is associated with a higher percentage of Ki67-positive cells in tumor tis-

sues. In in vitro culture models, the α-smooth muscle actin-positive CAFs enhance colony formation of luminal breast cancer cell lines, which is attenuated by OPN-neutralizing antibodies [43]. CAFs that are cultured with TAM-derived OPN significantly increase OPN expression, which is associated with enhanced proliferation, invasion, and migration of HCC cells [198]. In myofibroblasts, the signal adaptor MyD88, an essential component of TLR signaling, is activated in colitis-associated cancer [199]. MyD88 signaling in myofibroblasts increases the secretion of OPN, which promotes macrophage M2 polarization via activation of the STAT3/PPAR γ pathway. CAF-derived OPN may also contribute to tumor progression by increasing the stem cell population. In the stroma of human breast cancer, the increased expression of cyclin D1 is associated with poor outcomes [200]. Cyclin D1 can transform fibroblasts to CAFs that upregulate OPN expression, and then the OPN induces stem cell expansion. Indeed, the abundance of OPN is increased >30-fold in the stromal fibroblasts of patients with invasive breast cancer, associated with poor outcomes [200]. Similarly, CAF-secreted OPN promotes in vivo clonogenicity in colon cancer [201] and increases the cancer stem cell population via the OPN-CD44 axis in pancreatic carcinoma cells [110].

CAF also accelerates tumor progression indirectly through the OPN induction in cancer cells. CAF-derived IL-6 triggers the induction of OPN production in head and neck cancer cells, which accelerates the cancer cell proliferation, migration, invasion, tumor growth, and metastasis via the αvβ3 integrin-NF-kB signaling pathway [202]. Similarly, hepatic stellate cell-derived nuclear receptor member family 4 subgroup A number 2 (NR4A2), a transcription factor previously reported as a molecular switch between inflammation and cancer, induces OPN expression in intrahepatic cholangiocarcinoma cells [203]. The OPN activates Wnt/β-catenin signaling in the cancer cells and thereby promotes tumor progression [203]. These results suggest that CAFs may play pivotal roles in OPN-mediated tumor progression.

9. Diagnostic and Therapeutic Applications of OPN in Cancer

9.1. Potential Applications as a Biomarker

OPN is now recognized as the lead marker in several types of cancers, which is associated with tumor progression [37,68,204–207]. Serum OPN level and promoter polymorphism is correlated with the clinicopathological criteria of the patients with metastatic breast cancer, response to the treatment, progression-free survival, and overall survival in clinical trials (ClinicalTrials.gov identifier: NCT04274504).

OPN and laminin α4 chain are increased in the CSF samples from glioblastoma patients compared to those from non-brain tumor patients, and their levels are significantly correlated with tumor volume [208]. This result suggests that the levels of OPN and laminin α4 chain in CSF samples appear to be candidates as diagnostic markers for glioblastoma [208].

OPN is a promising diagnostic marker for HCC, and the level of serum OPN is already increased a year prior to HCC diagnosis [209]. Indeed, OPN is a comparable marker to α-fetoprotein (AFP), which is a serum biomarker widely used in the diagnosis of HCC, and the sensitivity of OPN is higher than that of AFP. The combination of OPN and AFP is able to elevate the sensitivity of the diagnosis as compared to AFP alone, especially in the early diagnosis of HCC [210]. Similarly, the combination of serum CEA and OPN improves the sensitivity of the diagnosis of NSCLC [211]. Combining four biomarkers, migration inhibitory factor, OPN, prolactin, and CA-125, can better detect ovarian cancer from healthy controls compared to CA-125 alone [212]. Furthermore, the combination of plasma CA-125, HE4, OPN, leptin, and prolactin surpasses each single marker in its diagnostic value to discriminate between benign and malignant ovarian tumors [213].

The NSCLC patients with the C/C genotype at nt −443 in the OPN promoter have a significantly higher incidence of bone metastasis development and significantly lower survival rates compared to the other two genotypes (C/T, T/T) [214]. This result sug-

gests that −443C/T polymorphism of OPN may be a potential predictive biomarker for bone metastasis.

Patients with chronic obstructive pulmonary disease (COPD) have an increased risk of lung cancer, and the coexistence of both diseases is associated with poor survival [215]. Nevertheless, the molecular mechanisms remain unclear. Therefore, it is important to identify potential pathological genes and pathways involved [215]. Miao et al. have shown that OPN expression levels are significantly higher in the NSCLC patients with COPD than in NSCLC patients. Thus, the upregulation of OPN may be associated with an increased risk of lung cancer patients having COPD and be a potential predictive biomarker for the disease.

OPN may be a biomarker associated with the response to cancer chemotherapy. Cetuximab, which is a monoclonal antibody that targets EGFR, is used for colorectal cancer treatment. Effective cetuximab treatment induces an increase in the IL-33 level and a decrease in the OPN level in the peripheral blood at the early stage. Moreover, the secretion of OPN is inhibited by IL-33 administration in cetuximab-treated peripheral blood mononuclear cells from the effective group patients [216]. These results suggest that IL-33 and OPN levels could be potential biomarkers of cetuximab treatment efficacy. Likewise, in patients with metastatic non-clear cell renal cell carcinoma, OPN is identified as a biomarker associated with poor prognosis during treatment with the receptor tyrosine kinase inhibitor, sunitinib, or the mTOR inhibitor, everolimus [217]. In addition, high baseline OPN levels are associated with a worse response to nivolumab, a humanized monoclonal antibody against PD-1, in patients with NSCLC. Patients above the cut-off value of OPN have a higher mortality rate as compared to the patients with low serum OPN [218]. Moreover, increased expression of OPN and a low density of $CD8^+$ T cells are significantly associated with an unfavorable response to 5-FU-based adjuvant chemotherapy in stage III colon cancer [219]. Thus, OPN may serve as a predictive biomarker for the response to chemotherapy and the biomarker analysis may facilitate personalized therapy.

Since a high concentration of OPN is found in healthy human blood and many cells secrete OPN, the sensitivity and the specificity of OPN as a cancer biomarker may be low in the early diagnosis. To overcome this shortcoming, the measurement of PTMs on OPN may be useful. As discussed in Section 6, OPN undergoes PTMs such as proteolytic processing, phosphorylation, and glycosylation, which are altered at different disease stages and associated with disease progression. Therefore, the validation of cancer-specific or tumor stage-specific OPN PTMs, which has distinct PTMs from the normal tissue-derived OPN, may be a promising approach for translation into the clinical settings.

9.2. Potential Applications as a Therapeutic Target

OPN is considered a promising therapeutic target for cancer, and several antibodies against OPN have been developed for the treatment, demonstrating favorable efficacy in animal models. The monoclonal antibody AOM1 (Pfizer Inc., New York, NY, USA), which was identified by a phage display technology, binds to the SVVYGLR sequence that is the binding site for α4β1, α4β7, and α9β1 integrins and is immediately adjacent to the RGD motif and thrombin cleavage site as well (Figure 2). AOM1 efficiently inhibits both binding of OPN to αvβ3 integrin and OPN cleavage by thrombin [220]. AOM1 also inhibits tumor growth in the metastatic lesions but not primary tumor growth in a metastatic mouse model of NSCLC [220]. Other neutralization monoclonal antibodies 100D3 and 100D6 are shown to block the binding of OPN to T cells, and significantly increase the cytotoxic effects of tumor-specific CTLs and suppress tumor growth [177]. Additionally, a humanized OPN antibody hu1A12, which recognizes $N^{212}APSD^{216}$, inhibits in vitro MDA-MB435S cancer cell adhesion, migration, and colony formation, as well as in vivo primary tumor growth and metastasis [221]. Although the efficacy against cancer remains to be elucidated, there are similar antibodies against OPN, C2K1, ASK8007 (Astellas Pharma Inc., Tokyo, Japan), and 23C3. The C2K1 and ASK8007 recognize the SVVYGLR sequence, while 23C3 recognizes the N-terminal region of OPN. However, it should be recognized that

the administration of ASK8007 showed no clinical improvement in rheumatoid arthritis patients and led to an accumulation of full-length OPN levels in plasma [222]. Thus, if ASK8007 is used in clinical trials for the treatment of cancer, removal of the accumulated full-length OPN may be necessary.

Farrokhi et al. have reported that the study using a stable isotope-labeled amino acid pulse-chase and mass spectrometry shows that OPN undergoes very rapid turnover in healthy human subjects [223]. Furthermore, their pharmacokinetic/pharmacodynamics models and simulation for potential anti-OPN antibody therapeutics reveal that achieving sufficient target coverage using conventional antibodies would not be feasible in humans, mostly due to the very rapid turnover of OPN, as well as the presence of a high concentration of OPN in plasma [223]. Therefore, therapeutic antibodies against OPN may be required to have more extended pharmacokinetics than conventional ones, and be administrated at high doses and with short dosing intervals [223]. Alternatively, the antibodies targeting integrin and CD44 receptors may be useful for OPN-targeted therapy for cancer [106,224,225].

Inhibitors that downregulate OPN expression may also be useful for cancer treatment. In mice models of mammary carcinoma, treatment of siRNA against OPN encapsulated in nanoparticles results in significant inhibition of tumor growth, accompanied by a significant reduction of OPN mRNA levels [226]. The bromodomain and extra-terminal domain (BET) protein family consists of four members, BRDT, BRD2, BRD3, and BRD4, and has tandem N-terminal bromodomains. BET proteins recognize acetylated lysine in histones and influence transcriptional activity and chromatin remodeling. Some small-molecule BET inhibitors are already under clinical trials for the treatment of cancers [227]. BET inhibitors target BRD4 and suppress OPN expression via transcriptional inactivation of NF-κB2, leading to impede melanoma cell proliferation, migration, and invasion [228]. Additionally, conophylline, a vinca alkaloid obtained from the leaves of *Ervatamia microphylla*, also inhibits HCC cell proliferation and tumor growth by suppressing the production of CAF-secreted cytokines such as IL6, IL8, CCL2, angiogenin, and OPN [229]. Conophylline treatment alone inhibits tumor growth, but when combined with sorafenib, the anti-tumor effect is enhanced compared with a single treatment with conophylline or sorafenib [229]. Another natural compound-based antioxidant and anti-inflammatory nutritional complement, ocoxin, also shows a reduced secretion of galectin-1, OPN, CCL5, and CCL9 from melanoma tumor cells, as well as the reduced number of lung metastasis of melanoma cells [230].

Inactivation of OPN may be another strategy for cancer therapy. Follistatin-like protein 1 (FSTL-1) is a secreted glycoprotein and a critical developmental regulator of lung organogenesis, but its expression negatively correlates with poor clinical outcome in patients with NSCLC and with the metastatic potential of lung cancer cells [231]. Mechanistically, FSTL-1 directly binds to the un-cleaved form of OPN, restraining the proteolytic activation of OPN, which leads to inactivation of integrin/CD44-associated signaling [231]. Furthermore, the combination of low expression of FSTL1 and high expression of OPN predicts a poorer prognosis for patients with lung cancer [231]. These results suggest that the combination of FSTL1 and OPN levels might be a potential biomarker of lung cancer, and upregulation of FSTL1 could be a potential therapy for OPN-mediated lung cancer. Since thrombin proteolytic processing alters OPN functions and its receptor interaction, the inhibition of thrombin activity may be a potential therapeutic strategy for treating the OPN-mediated cancer. Indeed, treatment of thrombin inhibitor, dabigatran etexilate, suppresses OPN-mediated B16 melanoma growth [10]. Thus, suppression of OPN activity by inhibiting OPN signaling and proteolytic processing may be useful as a therapy to block tumor progression.

Immune checkpoint inhibitors are novel and successful immunotherapy drugs in many advanced cancers [232]. Immune checkpoint inhibitors targeting the PD-1/PD-L1 interaction have been developed and are currently in use for the treatment of many cancers; however, not all human cancers respond to the immune checkpoint inhibitor immunotherapy, and meaningful responses to the immune checkpoint inhibitors remain low [2,232].

Therefore, it is important to understand the molecular mechanisms to improve responses to the immunotherapy. As discussed in Section 7.2, OPN participates in regulation of PD-L1 expression and the ICOS–ICOSL interaction, which promote immune surveillance evasion. Thus, OPN blockade might become an attractive target for cancer immunotherapy.

Pancreatic cancer is refractory to immune checkpoint inhibitor immunotherapy. Lu et al. found that H3K4 methylation is highly enriched through the pancreatic tumor genome and OPN expression is upregulated by the increased H3K4 methylation in its promoter region [69]. WDR-5 is an adaptor protein required for H3K4me3-specific histone methyltransferase activity. Inhibition of WDR-5 significantly decreases the OPN protein level and enhances the efficacy of anti-PD-1 immunotherapy, which result in suppression of pancreatic tumor growth in mouse models [69]. These results indicate that inhibition of OPN signaling might enhance the efficacy of immune therapy targeting the PD1/PD-L1 interaction. In contrast, low-dose anti-VEGFR2 therapy is more effective in sensitizing breast cancer to anti-PD-1 therapy through upregulation of OPN and TGF-β expression [233]. Mechanistically, low-dose anti-VEGFR2 antibody treatment results in more robust immune cell infiltration and activation and promotes OPN secretion by $CD8^+$ T cells. The OPN induces TGF-β production in tumor cells, which in turn upregulates PD-1 expression on immune cells. Indeed, in patients with triple-negative breast cancer, higher OPN and TGF-β expressions correlate with an improved response to treatment with anti-PD-1 and low-dose anti-VEGFR2 antibodies [233]. Although the biological mechanisms that underlie these opposite functions of OPN in the immune system have not been fully elucidated, the exact effects of OPN on immune regulation and on the potential responses of tumors to immune checkpoint inhibitors require further investigation [233].

10. Conclusions

Recent studies have shown that the interaction between tumor cells and the TME promotes tumor progression. One of the TME components, OPN, is produced by various cells, including tumor cells, endothelial cells, immune cells, as well as fibroblast cells, within the TME, and plays a central role in tumor progression. OPN promotes tumor growth, tumor cell invasion, metastasis, EMT, drug-resistance, stemness, angiogenesis, and immune suppression through cell surface receptors such as integrins and CD44. A number of studies have suggested that the blockade of OPN/receptor signaling may be highly relevant for the development of new cancer treatments. Additionally, better understanding the mechanisms underlying the regulation of immune checkpoint and immune cells as well as CAFs by OPN may provide a new strategy for cancer treatment.

OPN is expressed in many normal cells and plays important roles in physiological processes, such as cell adhesion, migration, proliferation, survival, differentiation, and immune modulation. Accordingly, the anti-OPN drugs can target the OPN molecule not only in the tumor tissues but also in normal tissues, which may cause severe side effects because of the inhibitory effects on the physiological activities of OPN. Therefore, the development of drugs targeting tumor cell-specific OPN distinct from normal cell-specific OPN is required to avoid these issues. PTMs of proteins in tumor tissues are completely different from those in normal tissues because of the differential expression patterns of modified enzymes between the two tissues. Therefore, the combination of OPN and its PTMs may be targets for tumor-specific OPN. Furthermore, the tumor-specific OPN may be valuable tool for the target delivery system and visualization system of cancer location and extent. For example, the nanoparticles that are surface-decorated with antibodies and small molecules against tumor-specific OPN may be useful for drug delivery to tumor cells and visualization of tumor cells [234].

As discussed in this review, OPN is an extremely complex and confusing molecule, but also an attractive target for cancer treatment. Unfortunately, there are currently no clinical trials targeting OPN in cancer (https://clinicaltrials.gov/, accessed on 15 August 2022). However, understanding the pleiotropic roles and PTMs of OPN in tumor progression will give us the opportunity to treat currently incurable cancers.

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References

1. Sung, H.; Ferlay, J.; Siegel, R.L.; Laversanne, M.; Soerjomataram, I.; Jemal, A.; Bray, F. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA Cancer J. Clin.* **2021**, *71*, 209–249. [CrossRef] [PubMed]
2. Lee, J.B.; Ha, S.-J.; Kim, H.R. Clinical Insights into Novel Immune Checkpoint Inhibitors. *Front. Pharmacol.* **2021**, *12*, 681320. [CrossRef] [PubMed]
3. Quail, D.F.; Joyce, J.A. Microenvironmental regulation of tumor progression and metastasis. *Nat. Med.* **2013**, *19*, 1423–1437. [CrossRef] [PubMed]
4. Wei, R.; Liu, S.; Zhang, S.; Min, L.; Zhu, S. Cellular and Extracellular Components in Tumor Microenvironment and Their Application in Early Diagnosis of Cancers. *Anal. Cell. Pathol.* **2020**, *2020*, 6283796. [CrossRef]
5. Senger, D.R.; Wirth, D.F.; Hynes, R.O. Transformed mammalian cells secrete specific proteins and phosphoproteins. *Cell* **1979**, *16*, 885–893. [CrossRef]
6. Moorman, H.R.; Poschel, D.; Klement, J.D.; Lu, C.; Redd, P.S.; Liu, K. Osteopontin: A Key Regulator of Tumor Progression and Immunomodulation. *Cancers* **2020**, *12*, 3379. [CrossRef]
7. Pang, X.; Gong, K.; Zhang, X.; Wu, S.; Cui, Y.; Qian, B.-Z. Osteopontin as a multifaceted driver of bone metastasis and drug resistance. *Pharmacol. Res.* **2019**, *144*, 235–244. [CrossRef]
8. Kariya, Y.; Kanno, M.; Matsumoto-Morita, K.; Konno, M.; Yamaguchi, Y.; Hashimoto, Y. Osteopontin O-glycosylation contributes to its phosphorylation and cell-adhesion properties. *Biochem. J.* **2014**, *463*, 93–102. [CrossRef]
9. Kariya, Y.; Oyama, M.; Kariya, Y.; Hashimoto, Y. Phosphorylated Osteopontin Secreted from Cancer Cells Induces Cancer Cell Motility. *Biomolecules* **2021**, *11*, 1323. [CrossRef]
10. Peraramelli, S.; Zhou, Q.; Zhou, Q.; Wanko, B.; Zhao, L.; Nishimura, T.; Leung, T.H.; Mizuno, S.; Ito, M.; Myles, T.; et al. Thrombin cleavage of osteopontin initiates osteopontin's tumor-promoting activity. *J. Thromb. Haemost.* **2022**, *20*, 1256–1270. [CrossRef]
11. Minai-Tehrani, A.; Chang, S.-H.; Park, S.B.; Cho, M.-H. The O-glycosylation mutant osteopontin alters lung cancer cell growth and migration in vitro and in vivo. *Int. J. Mol. Med.* **2013**, *32*, 1137–1149. [CrossRef] [PubMed]
12. Kariya, Y.; Oyama, M.; Hashimoto, Y.; Gu, J.; Kariya, Y. β4-Integrin/PI3K Signaling Promotes Tumor Progression through the Galectin-3-N-Glycan Complex. *Mol. Cancer Res.* **2018**, *16*, 1024–1034. [CrossRef]
13. Schytte, G.N.; Christensen, B.; Bregenov, I.; Kjøge, K.; Scavenius, C.; Petersen, S.V.; Enghild, J.J.; Sørensen, E.S. FAM20C phosphorylation of the RGDSVVYGLR motif in osteopontin inhibits interaction with the αvβ3 integrin. *J. Cell. Biochem.* **2020**, *121*, 4809–4818. [CrossRef] [PubMed]
14. Shao, L.; Zhang, B.; Wang, L.; Wu, L.; Kan, Q.; Fan, K. MMP-9-cleaved osteopontin isoform mediates tumor immune escape by inducing expansion of myeloid-derived suppressor cells. *Biochem. Biophys. Res. Commun.* **2017**, *493*, 1478–1484. [CrossRef]
15. Virchow, R. *Cellular Pathology as Based upon Physiological and Pathological Histology*; J. B. Lippincott: Philadelphia, PA, USA, 1863.
16. Gerarduzzi, C.; Hartmann, U.; Leask, A.; Drobetsky, E. The Matrix Revolution: Matricellular Proteins and Restructuring of the Cancer Microenvironment. *Cancer Res.* **2020**, *80*, 2705–2717. [CrossRef] [PubMed]
17. Lamort, A.S.; Giopanou, I.; Psallidas, I.; Stathopoulos, G.T. Osteopontin as a Link between Inflammation and Cancer: The Thorax in the Spotlight. *Cells* **2019**, *8*, 815. [CrossRef] [PubMed]
18. Rosmus, D.-D.; Lange, C.; Ludwig, F.; Ajami, B.; Wieghofer, P. The Role of Osteopontin in Microglia Biology: Current Concepts and Future Perspectives. *Biomedicines* **2022**, *10*, 840. [CrossRef]
19. Singh, A.; Gill, G.; Kaur, H.; Amhmed, M.; Jakhu, H. Role of osteopontin in bone remodeling and orthodontic tooth movement: A review. *Prog. Orthod.* **2018**, *19*, 18. [CrossRef]
20. Kariya, Y.; Kariya, Y.; Saito, T.; Nishiyama, S.; Honda, T.; Tanaka, K.; Yoshida, M.; Fujihara, K.; Hashimoto, Y. Increased cerebrospinal fluid osteopontin levels and its involvement in macrophage infiltration in neuromyelitis optica. *BBA Clin.* **2015**, *3*, 126–134. [CrossRef]
21. Demmelmair, H.; Prell, C.; Timby, N.; Lönnerdal, B. Benefits of Lactoferrin, Osteopontin and Milk Fat Globule Membranes for Infants. *Nutrients* **2017**, *9*, 817. [CrossRef]
22. Yang, L.; Chen, J.H.; Cai, D.; Wang, L.Y.; Zha, X.L. Osteopontin and integrin are involved in cholesterol gallstone formation. *Med. Sci. Monit. Int. Med. J. Exp. Clin. Res.* **2012**, *18*, BR16–BR23. [CrossRef]

23. Kahles, F.; Findeisen, H.M.; Bruemmer, D. Osteopontin: A novel regulator at the cross roads of inflammation, obesity and diabetes. *Mol. Metab.* **2014**, *3*, 384–393. [CrossRef] [PubMed]
24. Sodek, J.; Ganss, B.; McKee, M.D. Osteopontin. Critical reviews in oral biology and medicine: An official publication of the American Association of Oral Biologists. *Crit. Rev. Oral Biol. Med.* **2000**, *11*, 279–303. [CrossRef] [PubMed]
25. Kazanecki, C.C.; Uzwiak, D.J.; Denhardt, D.T. Control of osteopontin signaling and function by post-translational phosphorylation and protein folding. *J. Cell. Biochem.* **2007**, *102*, 912–924. [CrossRef] [PubMed]
26. Yim, A.; Smith, C.; Brown, A.M. Osteopontin/secreted phosphoprotein-1 harnesses glial-, immune-, and neuronal cell ligand-receptor interactions to sense and regulate acute and chronic neuroinflammation. *Immunol. Rev.* **2022**. early view. [CrossRef]
27. Song, Z.; Chen, W.; Athavale, D.; Ge, X.; Desert, R.; Das, S.; Han, H.; Nieto, N. Osteopontin Takes Center Stage in Chronic Liver Disease. *Hepatology* **2021**, *73*, 1594–1608. [CrossRef]
28. Anan, G.; Yoneyama, T.; Noro, D.; Tobisawa, Y.; Hatakeyama, S.; Sutoh Yoneyama, M.; Yamamoto, H.; Imai, A.; Iwamura, H.; Kohada, Y.; et al. The Impact of Glycosylation of Osteopontin on Urinary Stone Formation. *Int. J. Mol. Sci.* **2020**, *21*, 93. [CrossRef]
29. Kurzbach, D.; Platzer, G.; Schwarz, T.C.; Henen, M.A.; Konrat, R.; Hinderberger, D. Cooperative Unfolding of Compact Conformations of the Intrinsically Disordered Protein Osteopontin. *Biochemistry* **2013**, *52*, 5167–5175. [CrossRef]
30. Wright, P.E.; Dyson, H.J. Intrinsically disordered proteins in cellular signalling and regulation. *Nat. Rev. Mol. Cell Biol.* **2015**, *16*, 18–29. [CrossRef]
31. Martinelli, A.; Lopes, F.; John, E.; Carlini, C.; Ligabue-Braun, R. Modulation of Disordered Proteins with a Focus on Neurodegenerative Diseases and Other Pathologies. *Int. J. Mol. Sci.* **2019**, *20*, 1322. [CrossRef]
32. Gao, J.; Xu, D. Correlation between posttranslational modification and intrinsic disorder in protein. *Biocomputing* **2012**, 94–103. [CrossRef]
33. Zhou, J.; Zhao, S.; Dunker, A.K. Intrinsically Disordered Proteins Link Alternative Splicing and Post-translational Modifications to Complex Cell Signaling and Regulation. *J. Mol. Biol.* **2018**, *430*, 2342–2359. [CrossRef] [PubMed]
34. Gimba, E.; Brum, M.; Nestal De Moraes, G. Full-length osteopontin and its splice variants as modulators of chemoresistance and radioresistance (Review). *Int. J. Oncol.* **2019**, *54*, 420–430. [CrossRef] [PubMed]
35. Katagiri, Y.U.; Sleeman, J.; Fujii, H.; Herrlich, P.; Hotta, H.; Tanaka, K.; Chikuma, S.; Yagita, H.; Okumura, K.; Murakami, M.; et al. CD44 variants but not CD44s cooperate with beta1-containing integrins to permit cells to bind to osteopontin independently of arginine-glycine-aspartic acid, thereby stimulating cell motility and chemotaxis. *Cancer Res.* **1999**, *59*, 219–226. [PubMed]
36. Kanayama, M.; Xu, S.; Danzaki, K.; Gibson, J.R.; Inoue, M.; Gregory, S.G.; Shinohara, M.L. Skewing of the population balance of lymphoid and myeloid cells by secreted and intracellular osteopontin. *Nat. Immunol.* **2017**, *18*, 973–984. [CrossRef]
37. Liu, Y.; Ye, G.; Dong, B.; Huang, L.; Zhang, C.; Sheng, Y.; Wu, B.; Han, L.; Wu, C.; Qi, Y. A pan-cancer analysis of the oncogenic role of secreted phosphoprotein 1 (SPP1) in human cancers. *Ann. Transl. Med.* **2022**, *10*, 279. [CrossRef]
38. Subraman, V.; Thiyagarajan, M.; Malathi, N.; Rajan, S.T. OPN—Revisited. *J. Clin. Diagn. Res.* **2015**, *9*, ZE10–ZE13. [CrossRef]
39. Hao, C.; Cui, Y.; Owen, S.; Li, W.; Cheng, S.; Jiang, W.G. Human osteopontin: Potential clinical applications in cancer (Review). *Int. J. Mol. Med.* **2017**, *39*, 1327–1337. [CrossRef]
40. Chen, P.; Zhao, D.; Li, J.; Liang, X.; Li, J.; Chang, A.; Henry, V.K.; Lan, Z.; Spring, D.J.; Rao, G.; et al. Symbiotic Macrophage-Glioma Cell Interactions Reveal Synthetic Lethality in PTEN-Null Glioma. *Cancer Cell* **2019**, *35*, 868–884. [CrossRef]
41. Zhang, L.; Li, Z.; Skrzypczynska, K.M.; Fang, Q.; Zhang, W.; O'Brien, S.A.; He, Y.; Wang, L.; Zhang, Q.; Kim, A.; et al. Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. *Cell* **2020**, *181*, 442–459. [CrossRef]
42. Yonemitsu, K.; Miyasato, Y.; Shiota, T.; Shinchi, Y.; Fujiwara, Y.; Hosaka, S.; Yamamoto, Y.; Komohara, Y. Soluble Factors Involved in Cancer Cell-Macrophage Interaction Promote Breast Cancer Growth. *Anticancer Res.* **2021**, *41*, 4249–4258. [CrossRef] [PubMed]
43. Muchlińska, A.; Nagel, A.; Popęda, M.; Szade, J.; Niemira, M.; Zieliński, J.; Skokowski, J.; Bednarz-Knoll, N.; Żaczek, A.J. Alpha-smooth muscle actin-positive cancer-associated fibroblasts secreting osteopontin promote growth of luminal breast cancer. *Cell. Mol. Biol. Lett.* **2022**, *27*, 45. [CrossRef]
44. Shiomi, A.; Kusuhara, M.; Sugino, T.; Sugiura, T.; Ohshima, K.; Nagashima, T.; Urakami, K.; Serizawa, M.; Saya, H.; Yamaguchi, K. Comprehensive genomic analysis contrasting primary colorectal cancer and matched liver metastases. *Oncol. Lett.* **2021**, *21*, 466. [CrossRef] [PubMed]
45. Briones-Orta, M.A.; Avendaño-Vázquez, S.E.; Aparicio-Bautista, D.I.; Coombes, J.D.; Weber, G.F.; Syn, W.-K. Osteopontin splice variants and polymorphisms in cancer progression and prognosis. *Biochim. Biophys. Acta BBA Rev. Cancer* **2017**, *1868*, 93–108.A. [CrossRef] [PubMed]
46. Adams, C.R.; Htwe, H.H.; Marsh, T.; Wang, A.L.; Montoya, M.L.; Subbaraj, L.; Tward, A.D.; Bardeesy, N.; Perera, R.M. Transcriptional control of subtype switching ensures adaptation and growth of pancreatic cancer. *eLife* **2019**, *8*, e45313. [CrossRef]
47. Feng, Y.-H.; Su, Y.-C.; Lin, S.-F.; Lin, P.-R.; Wu, C.-L.; Tung, C.-L.; Li, C.-F.; Shieh, G.-S.; Shiau, A.-L. Oct4 upregulates osteopontin via Egr1 and is associated with poor outcome in human lung cancer. *BMC Cancer* **2019**, *19*, 791. [CrossRef]
48. Krstic, M.; Hassan, H.M.; Kolendowski, B.; Hague, M.N.; Anborgh, P.H.; Postenka, C.O.; Torchia, J.; Chambers, A.F.; Tuck, A.B. Isoform-specific promotion of breast cancer tumorigenicity by TBX3 involves induction of angiogenesis. *Lab. Investig.* **2020**, *100*, 400–413. [CrossRef]
49. Liu, K.; Hu, H.; Jiang, H.; Zhang, H.; Gong, S.; Wei, D.; Yu, Z. RUNX1 promotes MAPK signaling to increase tumor progression and metastasis via OPN in head and neck cancer. *Carcinogenesis* **2021**, *42*, 414–422. [CrossRef]

50. Deiana, M.; Dalle Carbonare, L.; Serena, M.; Cheri, S.; Mutascio, S.; Gandini, A.; Innamorati, G.; Lorenzi, P.; Cumerlato, M.; Bertacco, J.; et al. A Potential Role of RUNX2- RUNT Domain in Modulating the Expression of Genes Involved in Bone Metastases: An In Vitro Study with Melanoma Cells. *Cells* **2020**, *9*, 751. [CrossRef]
51. Whittle, M.C.; Izeradjene, K.; Rani, P.G.; Feng, L.; Carlson, M.A.; DelGiorno, K.E.; Wood, L.D.; Goggins, M.; Hruban, R.H.; Chang, A.E.; et al. RUNX3 Controls a Metastatic Switch in Pancreatic Ductal Adenocarcinoma. *Cell* **2015**, *161*, 1345–1360. [CrossRef]
52. Amilca-Seba, K.; Tan, T.Z.; Thiery, J.-P.; Louadj, L.; Thouroude, S.; Bouygues, A.; Sabbah, M.; Larsen, A.K.; Denis, J.A. Osteopontin (OPN/SPP1), a Mediator of Tumor Progression, Is Regulated by the Mesenchymal Transcription Factor Slug/SNAI2 in Colorectal Cancer (CRC). *Cells* **2022**, *11*, 1808. [CrossRef] [PubMed]
53. Thomann, S.; Weiler, S.M.E.; Marquard, S.; Rose, F.; Ball, C.R.; Tóth, M.; Wei, T.; Sticht, C.; Fritzsche, S.; Roessler, S.; et al. YAP Orchestrates Heterotypic Endothelial Cell Communication via HGF/c-MET Signaling in Liver Tumorigenesis. *Cancer Res.* **2020**, *80*, 5502–5514. [CrossRef] [PubMed]
54. Klement, J.D.; Paschall, A.V.; Redd, P.S.; Ibrahim, M.L.; Lu, C.; Yang, D.; Celis, E.; Abrams, S.I.; Ozato, K.; Liu, K. An osteopontin/CD44 immune checkpoint controls $CD8^+$ T cell activation and tumor immune evasion. *J. Clin. Investig.* **2018**, *128*, 5549–5560. [CrossRef] [PubMed]
55. Sun, H.; Wang, C.; Hu, B.; Gao, X.; Zou, T.; Luo, Q.; Chen, M.; Fu, Y.; Sheng, Y.; Zhang, K.; et al. Exosomal S100A4 derived from highly metastatic hepatocellular carcinoma cells promotes metastasis by activating STAT3. *Signal Transduct. Target. Ther.* **2021**, *6*, 187. [CrossRef] [PubMed]
56. Li, Y.; Guo, S.; Zhao, K.; Conrad, C.; Driescher, C.; Rothbart, V.; Schlomann, U.; Guerreiro, H.; Bopp, M.H.; König, A.; et al. ADAM8 affects glioblastoma progression by regulating osteopontin-mediated angiogenesis. *Biol. Chem.* **2021**, *402*, 195–206. [CrossRef]
57. Gan, N.; Zou, S.; Hang, W.; Yang, D.; Zhang, X.; Yin, Y. Osteopontin is Critical for Hyperactive mTOR-Induced Tumorigenesis in Oral Squamous Cell Carcinoma. *J. Cancer* **2017**, *8*, 1362–1370. [CrossRef]
58. Schulz, A.; Gorodetska, I.; Behrendt, R.; Fuessel, S.; Erdmann, K.; Foerster, S.; Datta, K.; Mayr, T.; Dubrovska, A.; Muders, M.H. Linking NRP2 With EMT and Chemoradioresistance in Bladder Cancer. *Front. Oncol.* **2020**, *9*, 1461. [CrossRef]
59. Yang, Y.-F.; Chang, Y.-C.; Jan, Y.-H.; Yang, C.-J.; Huang, M.-S.; Hsiao, M. Squalene synthase promotes the invasion of lung cancer cells via the osteopontin/ERK pathway. *Oncogenesis* **2020**, *9*, 78. [CrossRef]
60. Curtis, K.J.; Schiavi, J.; Mc Garrigle, M.J.; Kumar, V.; McNamara, L.M.; Niebur, G.L. Mechanical stimuli and matrix properties modulate cancer spheroid growth in three-dimensional gelatin culture. *J. R. Soc. Interface* **2020**, *17*, 20200568. [CrossRef]
61. Kolb, A.; Kleeff, J.; Guweidhi, A.; Esposito, I.; Giese, N.A.; Adwan, H.; Giese, T.; Büchler, M.W.; Berger, M.R.; Friess, H. Osteopontin influences the invasiveness of pancreatic cancer cells and is increased in neoplastic and inflammatory conditions. *Cancer Biol. Ther.* **2005**, *4*, 740–746. [CrossRef]
62. Chang, J.; Bhasin, S.S.; Bielenberg, D.R.; Sukhatme, V.P.; Bhasin, M.; Huang, S.; Kieran, M.W.; Panigrahy, D. Chemotherapy-generated cell debris stimulates colon carcinoma tumor growth via osteopontin. *FASEB J.* **2019**, *33*, 114–125. [CrossRef] [PubMed]
63. Wang, S.-Y.; Chen, C.-L.; Hu, Y.-C.; Chi, Y.; Huang, Y.-H.; Su, C.-W.; Jeng, W.-J.; Liang, Y.-J.; Wu, J.-C. High Expression of MicroRNA-196a is Associated with Progression of Hepatocellular Carcinoma in Younger Patients. *Cancers* **2019**, *11*, 1549. [CrossRef] [PubMed]
64. Marisetty, A.; Wei, J.; Kong, L.-Y.; Ott, M.; Fang, D.; Sabbagh, A.; Heimberger, A.B. MiR-181 Family Modulates Osteopontin in Glioblastoma Multiforme. *Cancers* **2020**, *12*, 3813. [CrossRef] [PubMed]
65. Taipaleenmäki, H.; Farina, N.H.; van Wijnen, A.J.; Stein, J.L.; Hesse, E.; Stein, G.S.; Lian, J.B. Antagonizing miR-218-5p attenuates Wnt signaling and reduces metastatic bone disease of triple negative breast cancer cells. *Oncotarget* **2016**, *7*, 79032–79046. [CrossRef] [PubMed]
66. Colden, M.; Dar, A.A.; Saini, S.; Dahiya, P.V.; Shahryari, V.; Yamamura, S.; Tanaka, Y.; Stein, G.; Dahiya, R.; Majid, S. MicroRNA-466 inhibits tumor growth and bone metastasis in prostate cancer by direct regulation of osteogenic transcription factor RUNX2. *Cell Death Dis.* **2017**, *8*, e2572. [CrossRef] [PubMed]
67. Qiu, C.; Li, C.; Zheng, Q.; Fang, S.; Xu, J.; Wang, H.; Guo, H. Metformin suppresses lung adenocarcinoma by downregulating long non-coding RNA (lncRNA) AFAP1-AS1 and secreted phosphoprotein 1 (SPP1) while upregulating miR-3163. *Bioengineered* **2022**, *13*, 11987–12002. [CrossRef] [PubMed]
68. Amilca-Seba, K.; Sabbah, M.; Larsen, A.K.; Denis, J.A. Osteopontin as a Regulator of Colorectal Cancer Progression and Its Clinical Applications. *Cancers* **2021**, *13*, 3793. [CrossRef] [PubMed]
69. Lu, C.; Liu, Z.; Klement, J.D.; Yang, D.; Merting, A.D.; Poschel, D.; Albers, T.; Waller, J.L.; Shi, H.; Liu, K. WDR5-H3K4me3 epigenetic axis regulates OPN expression to compensate PD-L1 function to promote pancreatic cancer immune escape. *J. Immunother. Cancer* **2021**, *9*, e002624. [CrossRef]
70. Giopanou, I.; Kanellakis, N.I.; Giannou, A.D.; Lilis, I.; Marazioti, A.; Spella, M.; Papaleonidopoulos, V.; Simoes, D.C.M.; Zazara, D.E.; Agalioti, T.; et al. Osteopontin drives KRAS-mutant lung adenocarcinoma. *Carcinogenesis* **2020**, *41*, 1134–1144. [CrossRef] [PubMed]
71. Wu, Q.; Li, L.; Miao, C.; Hasnat, M.; Sun, L.; Jiang, Z.; Zhang, L. Osteopontin promotes hepatocellular carcinoma progression through inducing JAK2/STAT3/NOX1-mediated ROS production. *Cell Death Dis.* **2022**, *13*, 341. [CrossRef]
72. Cao, J.; Li, J.; Sun, L.; Qin, T.; Xiao, Y.; Chen, K.; Qian, W.; Duan, W.; Lei, J.; Ma, J.; et al. Hypoxia-driven paracrine osteopontin/integrin αvβ3 signaling promotes pancreatic cancer cell epithelial–mesenchymal transition and cancer stem cell-like properties by modulating forkhead box protein M1. *Mol. Oncol.* **2019**, *13*, 228–245. [CrossRef] [PubMed]

73. Luo, X.; Ruhland, M.K.; Pazolli, E.; Lind, A.C.; Stewart, S.A. Osteopontin Stimulates Preneoplastic Cellular Proliferation Through Activation of the MAPK Pathway. *Mol. Cancer Res.* **2011**, *9*, 1018–1029. [CrossRef] [PubMed]
74. Qian, J.; LeSavage, B.L.; Hubka, K.M.; Ma, C.; Natarajan, S.; Eggold, J.T.; Xiao, Y.; Fuh, K.C.; Krishnan, V.; Enejder, A.; et al. Cancer-associated mesothelial cells promote ovarian cancer chemoresistance through paracrine osteopontin signaling. *J. Clin. Investig.* **2021**, *131*, e146186. [CrossRef]
75. Cao, L.; Fan, X.; Jing, W.; Liang, Y.; Chen, R.; Liu, Y.; Zhu, M.; Jia, R.; Wang, H.; Zhang, X.; et al. Osteopontin promotes a cancer stem cell-like phenotype in hepatocellular carcinoma cells via an integrin-NF-κB-HIF-1α pathway. *Oncotarget* **2015**, *6*, 6627–6640. [CrossRef] [PubMed]
76. Rao, G.; Wang, H.; Li, B.; Huang, L.; Xue, D.; Wang, X.; Jin, H.; Wang, J.; Zhu, Y.; Lu, Y.; et al. Reciprocal Interactions between Tumor-Associated Macrophages and CD44-Positive Cancer Cells via Osteopontin/CD44 Promote Tumorigenicity in Colorectal Cancer. *Clin. Cancer Res.* **2013**, *19*, 785–797. [CrossRef] [PubMed]
77. Huang, R.-H.; Quan, Y.-J.; Chen, J.-H.; Wang, T.-F.; Xu, M.; Ye, M.; Yuan, H.; Zhang, C.-J.; Liu, X.-J.; Min, Z.-J. Osteopontin Promotes Cell Migration and Invasion, and Inhibits Apoptosis and Autophagy in Colorectal Cancer by activating the p38 MAPK Signaling Pathway. *Cell. Physiol. Biochem.* **2017**, *41*, 1851–1864. [CrossRef]
78. Ahmed, M.; Sottnik, J.L.; Dancik, G.M.; Sahu, D.; Hansel, D.E.; Theodorescu, D.; Schwartz, M.A. An Osteopontin/CD44 Axis in RhoGDI2-Mediated Metastasis Suppression. *Cancer Cell* **2016**, *30*, 432–443. [CrossRef]
79. Gupta, A.; Zhou, C.; Chellaiah, M. Osteopontin and MMP9: Associations with VEGF Expression/Secretion and Angiogenesis in PC3 Prostate Cancer Cells. *Cancers* **2013**, *5*, 617–638. [CrossRef]
80. Pitarresi, J.R.; Norgard, R.J.; Chiarella, A.M.; Suzuki, K.; Bakir, B.; Sahu, V.; Li, J.; Zhao, J.; Marchand, B.; Wengyn, M.D.; et al. PTHrP Drives Pancreatic Cancer Growth and Metastasis and Reveals a New Therapeutic Vulnerability. *Cancer Discov.* **2021**, *11*, 1774–1791. [CrossRef]
81. Liu, H.; Wei, S.; Zhang, L.; Yuan, C.; Duan, Y.; Wang, Q. Secreted Phosphoprotein 1 Promotes the Development of Small Cell Lung Cancer Cells by Inhibiting Autophagy and Apoptosis. *Pathol. Oncol. Res.* **2019**, *25*, 1487–1495. [CrossRef]
82. Napoli, S.; Scuderi, C.; Gattuso, G.; Di Bella, V.; Candido, S.; Basile, M.S.; Libra, M.; Falzone, L. Functional Roles of Matrix Metalloproteinases and Their Inhibitors in Melanoma. *Cells* **2020**, *9*, 1151. [CrossRef] [PubMed]
83. Chen, R.X.; Xia, Y.H.; Xue, T.C.; Zhang, H.; Ye, S.L. Down-regulation of osteopontin inhibits metastasis of hepatocellular carcinoma cells via a mechanism involving MMP-2 and uPA. *Oncol. Rep.* **2011**, *25*, 803–808. [CrossRef] [PubMed]
84. Kechagia, J.Z.; Ivaska, J.; Roca-Cusachs, P. Integrins as biomechanical sensors of the microenvironment. *Nat. Rev. Mol. Cell Biol.* **2019**, *20*, 457–473. [CrossRef] [PubMed]
85. Kariya, Y.; Kariya, Y.; Gu, J. Roles of Integrin α6β4 Glycosylation in Cancer. *Cancers* **2017**, *9*, 79. [CrossRef] [PubMed]
86. Cooper, J.; Giancotti, F.G. Integrin Signaling in Cancer: Mechanotransduction, Stemness, Epithelial Plasticity, and Therapeutic Resistance. *Cancer Cell* **2019**, *35*, 347–367. [CrossRef]
87. Ludwig, B.S.; Kessler, H.; Kossatz, S.; Reuning, U. RGD-Binding Integrins Revisited: How Recently Discovered Functions and Novel Synthetic Ligands (Re-)Shape an Ever-Evolving Field. *Cancers* **2021**, *13*, 1711. [CrossRef]
88. Desgrosellier, J.S.; Cheresh, D.A. Integrins in cancer: Biological implications and therapeutic opportunities. *Nat. Rev. Cancer* **2010**, *10*, 9–22. [CrossRef]
89. Vogetseder, A.; Thies, S.; Ingold, B.; Roth, P.; Weller, M.; Schraml, P.; Goodman, S.L.; Moch, H. αv-Integrin isoform expression in primary human tumors and brain metastases. *Int. J. Cancer* **2013**, *133*, 2362–2371. [CrossRef]
90. Avraamides, C.J.; Garmy-Susini, B.; Varner, J.A. Integrins in angiogenesis and lymphangiogenesis. *Nat. Rev. Cancer* **2008**, *8*, 604–617. [CrossRef]
91. Böger, C.; Warneke, V.S.; Behrens, H.-M.; Kalthoff, H.; Goodman, S.L.; Becker, T.; Röcken, C. Integrins αvβ3 and αvβ5 as prognostic, diagnostic, and therapeutic targets in gastric cancer. *Gastric Cancer* **2015**, *18*, 784–795. [CrossRef]
92. Baiula, M.; Spampinato, S.; Gentilucci, L.; Tolomelli, A. Novel Ligands Targeting α4β1 Integrin: Therapeutic Applications and Perspectives. *Front. Chem.* **2019**, *7*, 489. [CrossRef] [PubMed]
93. LaFoya, B.; Munroe, J.A.; Miyamoto, A.; Detweiler, M.A.; Crow, J.J.; Gazdik, T.; Albig, A.R. Beyond the Matrix: The Many Non-ECM Ligands for Integrins. *Int. J. Mol. Sci.* **2018**, *19*, 449. [CrossRef] [PubMed]
94. Taooka, Y.; Chen, J.; Yednock, T.; Sheppard, D. The integrin alpha9beta1 mediates adhesion to activated endothelial cells and transendothelial neutrophil migration through interaction with vascular cell adhesion molecule-1. *J. Cell Biol.* **1999**, *145*, 413–420. [CrossRef] [PubMed]
95. Kale, S.; Raja, R.; Thorat, D.; Soundararajan, G.; Patil, T.V.; Kundu, G.C. Osteopontin signaling upregulates cyclooxygenase-2 expression in tumor-associated macrophages leading to enhanced angiogenesis and melanoma growth via α9β1 integrin. *Oncogene* **2014**, *33*, 2295–2306. [CrossRef] [PubMed]
96. Krishn, S.R.; Salem, I.; Quaglia, F.; Naranjo, N.M.; Agarwal, E.; Liu, Q.; Sarker, S.; Kopenhaver, J.; McCue, P.A.; Weinreb, P.H.; et al. The αvβ6 integrin in cancer cell-derived small extracellular vesicles enhances angiogenesis. *J. Extracell. Vesicles* **2020**, *9*, 1763594. [CrossRef]
97. Oyama, M.; Kariya, Y.; Kariya, Y.; Matsumoto, K.; Kanno, M.; Yamaguchi, Y.; Hashimoto, Y. Biological role of site-specific O-glycosylation in cell adhesion activity and phosphorylation of osteopontin. *Biochem. J.* **2018**, *475*, 1583–1595. [CrossRef]
98. Fan, C.S.; Chen, W.S.; Chen, L.L.; Chen, C.C.; Hsu, Y.T.; Chua, K.V.; Wang, H.D.; Huang, T.S. Osteopontin-integrin engagement induces HIF-1α-TCF12-mediated endothelial-mesenchymal transition to exacerbate colorectal cancer. *Oncotarget* **2018**, *9*, 4998–5015. [CrossRef]

99. Hsieh, I.S.; Huang, W.H.; Liou, H.C.; Chuang, W.J.; Yang, R.S.; Fu, W.M. Upregulation of drug transporter expression by osteopontin in prostate cancer cells. *Mol. Pharmacol.* **2013**, *83*, 968–977. [CrossRef]
100. Lu, C.; Fang, S.; Weng, Q.; Lv, X.; Meng, M.; Zhu, J.; Zheng, L.; Hu, Y.; Gao, Y.; Wu, X.; et al. Integrated analysis reveals critical glycolytic regulators in hepatocellular carcinoma. *Cell Commun. Signal.* **2020**, *18*, 97. [CrossRef]
101. Che, P.; Yu, L.; Friedman, G.K.; Wang, M.; Ke, X.; Wang, H.; Zhang, W.; Nabors, B.; Ding, Q.; Han, X. Integrin αvβ3 Engagement Regulates Glucose Metabolism and Migration through Focal Adhesion Kinase (FAK) and Protein Arginine Methyltransferase 5 (PRMT5) in Glioblastoma Cells. *Cancers* **2021**, *13*, 1111. [CrossRef]
102. Luengo, A.; Gui, D.Y.; Vander Heiden, M.G. Targeting Metabolism for Cancer Therapy. *Cell Chem. Biol.* **2017**, *24*, 1161–1180. [CrossRef] [PubMed]
103. Park, J.H.; Pyun, W.Y.; Park, H.W. Cancer Metabolism: Phenotype, Signaling and Therapeutic Targets. *Cells* **2020**, *9*, 2308. [CrossRef] [PubMed]
104. Shi, Z.; Wang, B.; Chihanga, T.; Kennedy, M.A.; Weber, G.F. Energy Metabolism during Anchorage-Independence. Induction by Osteopontin-c. *PLoS ONE* **2014**, *9*, e105675. [CrossRef] [PubMed]
105. Senbanjo, L.T.; Chellaiah, M.A. CD44: A Multifunctional Cell Surface Adhesion Receptor Is a Regulator of Progression and Metastasis of Cancer Cells. *Front. Cell Dev. Biol.* **2017**, *5*, 18. [CrossRef] [PubMed]
106. Hassn Mesrati, M.; Syafruddin, S.E.; Mohtar, M.A.; Syahir, A. CD44: A Multifunctional Mediator of Cancer Progression. *Biomolecules* **2021**, *11*, 1850. [CrossRef]
107. Fnu, G.; Agrawal, P.; Kundu, G.C.; Weber, G.F. Structural Constraint of Osteopontin Facilitates Efficient Binding to CD44. *Biomolecules* **2021**, *11*, 813. [CrossRef]
108. Weber, G.F.; Ashkar, S.; Glimcher, M.J.; Cantor, H. Receptor-ligand interaction between CD44 and osteopontin (Eta-1). *Science* **1996**, *271*, 509–512. [CrossRef]
109. Yan, Y.; Zuo, X.; Wei, D. Concise Review: Emerging Role of CD44 in Cancer Stem Cells: A Promising Biomarker and Therapeutic Target. *Stem Cells Transl. Med.* **2015**, *4*, 1033–1043. [CrossRef]
110. Nallasamy, P.; Nimmakayala, R.K.; Karmakar, S.; Leon, F.; Seshacharyulu, P.; Lakshmanan, I.; Rachagani, S.; Mallya, K.; Zhang, C.; Ly, Q.P.; et al. Pancreatic Tumor Microenvironment Factor Promotes Cancer Stemness via SPP1–CD44 Axis. *Gastroenterology* **2021**, *161*, 1998–2013. [CrossRef]
111. Pietras, A.; Katz, A.M.; Ekström, E.J.; Wee, B.; Halliday, J.J.; Pitter, K.L.; Werbeck, J.L.; Amankulor, N.M.; Huse, J.T.; Holland, E.C. Osteopontin-CD44 Signaling in the Glioma Perivascular Niche Enhances Cancer Stem Cell Phenotypes and Promotes Aggressive Tumor Growth. *Cell Stem Cell* **2014**, *14*, 357–369. [CrossRef]
112. Viana, B.P.P.B.; Gomes, A.V.P.; Gimba, E.R.P.; Ferreira, L.B. Osteopontin Expression in Thyroid Cancer: Deciphering EMT-Related Molecular Mechanisms. *Biomedicines* **2021**, *9*, 1372. [CrossRef] [PubMed]
113. Kothari, A.; Arffa, M.; Chang, V.; Blackwell, R.; Syn, W.-K.; Zhang, J.; Mi, Z.; Kuo, P. Osteopontin—A Master Regulator of Epithelial-Mesenchymal Transition. *J. Clin. Med.* **2016**, *5*, 39. [CrossRef] [PubMed]
114. Aiello, N.M.; Kang, Y. Context-dependent EMT programs in cancer metastasis. *J. Exp. Med.* **2019**, *216*, 1016–1026. [CrossRef] [PubMed]
115. Bakir, B.; Chiarella, A.M.; Pitarresi, J.R.; Rustgi, A.K. EMT, MET, Plasticity, and Tumor Metastasis. *Trends Cell Biol.* **2020**, *30*, 764–776. [CrossRef] [PubMed]
116. Shi, L.; Hou, J.; Wang, L.; Fu, H.; Zhang, Y.; Song, Y.; Wang, X. Regulatory roles of osteopontin in human lung cancer cell epithelial-to-mesenchymal transitions and responses. *Clin. Transl. Med.* **2021**, *11*, e486. [CrossRef]
117. Hao, C.; Cui, Y.; Chang, S.; Huang, J.; Birkin, E.; Hu, M.; Zhi, X.; Li, W.; Zhang, L.; Cheng, S.; et al. OPN promotes the aggressiveness of non-small-cell lung cancer cells through the activation of the RON tyrosine kinase. *Sci. Rep.* **2019**, *9*, 18101. [CrossRef]
118. Jia, R.; Liang, Y.; Chen, R.; Liu, G.; Wang, H.; Tang, M.; Zhou, X.; Wang, H.; Yang, Y.; Wei, H.; et al. Osteopontin facilitates tumor metastasis by regulating epithelial–mesenchymal plasticity. *Cell Death Dis.* **2016**, *7*, e2564. [CrossRef]
119. Saitoh, M. Involvement of partial EMT in cancer progression. *J. Biochem.* **2018**, *164*, 257–264. [CrossRef]
120. Kariya, Y.; Oyama, M.; Suzuki, T.; Kariya, Y. αvβ3 Integrin induces partial EMT independent of TGF-β signaling. *Commun. Biol.* **2021**, *4*, 490. [CrossRef]
121. Ding, K.U.N.; Fan, L.U.; Chen, S.; Wang, Y.; Yu, H.; Sun, Y.; Yu, J.; Wang, L.I.; Liu, X.; Liu, Y. Overexpression of osteopontin promotes resistance to cisplatin treatment in HCC. *Oncol. Rep.* **2015**, *34*, 3297–3303. [CrossRef]
122. Ng, L.; Wan, T.; Chow, A.; Iyer, D.; Man, J.; Chen, G.; Yau, T.C.-C.; Lo, O.; Foo, C.-C.; Poon, J.T.-C.; et al. Osteopontin Overexpression Induced Tumor Progression and Chemoresistance to Oxaliplatin through Induction of Stem-Like Properties in Human Colorectal Cancer. *Stem Cells Int.* **2015**, *2015*, 247892. [CrossRef] [PubMed]
123. Qian, C.; Li, P.; Yan, W.E.I.; Shi, L.E.I.; Zhang, J.; Wang, Y.; Liu, H.; You, Y. Downregulation of osteopontin enhances the sensitivity of glioma U251 cells to temozolomide and cisplatin by targeting the NF-κB/Bcl-2 pathway. *Mol. Med. Rep.* **2015**, *11*, 1951–1955. [CrossRef] [PubMed]
124. Insua-Rodríguez, J.; Pein, M.; Hongu, T.; Meier, J.; Descot, A.; Lowy, C.M.; De Braekeleer, E.; Sinn, H.P.; Spaich, S.; Sütterlin, M.; et al. Stress signaling in breast cancer cells induces matrix components that promote chemoresistant metastasis. *EMBO Mol. Med.* **2018**, *10*, e9003. [CrossRef] [PubMed]
125. Yang, M.-C.; Wang, H.-C.; Hou, Y.-C.; Tung, H.-L.; Chiu, T.-J.; Shan, Y.-S. Blockade of autophagy reduces pancreatic cancer stem cell activity and potentiates the tumoricidal effect of gemcitabine. *Mol. Cancer* **2015**, *14*, 179. [CrossRef]

126. Liu, G.; Fan, X.; Tang, M.; Chen, R.; Wang, H.; Jia, R.; Zhou, X.; Jing, W.; Wang, H.; Yang, Y.; et al. Osteopontin induces autophagy to promote chemo-resistance in human hepatocellular carcinoma cells. *Cancer Lett.* **2016**, *383*, 171–182. [CrossRef]
127. Fu, Y.; Zhang, Y.; Lei, Z.; Liu, T.; Cai, T.; Wang, A.; Du, W.; Zeng, Y.; Zhu, J.; Liu, Z.; et al. Abnormally activated OPN/integrin αVβ3/FAK signalling is responsible for EGFR-TKI resistance in EGFR mutant non-small-cell lung cancer. *J. Hematol. Oncol.* **2020**, *13*, 169. [CrossRef]
128. Zhang, H.; Wang, R.; Wang, M.; Luo, J.; Liu, C. Inhibition of osteopontin overcomes acquired resistance to afatinib in EGFR-mutant non-small-cell lung cancer. *Transl. Cancer Res.* **2020**, *9*, 754–762. [CrossRef]
129. Wang, X.; Zhang, F.; Yang, X.; Xue, M.; Li, X.; Gao, Y.; Liu, L. Secreted Phosphoprotein 1 (SPP1) Contributes to Second-Generation EGFR Tyrosine Kinase Inhibitor Resistance in Non-Small Cell Lung Cancer. *Oncol. Res. Featur. Preclin. Clin. Cancer Ther.* **2019**, *27*, 871–877. [CrossRef]
130. Mansoori, B.; Mohammadi, A.; Davudian, S.; Shirjang, S.; Baradaran, B. The Different Mechanisms of Cancer Drug Resistance: A Brief Review. *Adv. Pharm. Bull.* **2017**, *7*, 339–348. [CrossRef]
131. Graessmann, M.; Berg, B.; Fuchs, B.; Klein, A.; Graessmann, A. Chemotherapy resistance of mouse WAP-SVT/t breast cancer cells is mediated by osteopontin, inhibiting apoptosis downstream of caspase-3. *Oncogene* **2007**, *26*, 2840–2850. [CrossRef]
132. Horala, A.; Swiatly, A.; Matysiak, J.; Banach, P.; Nowak-Markwitz, E.; Kokot, Z. Diagnostic Value of Serum Angiogenesis Markers in Ovarian Cancer Using Multiplex Immunoassay. *Int. J. Mol. Sci.* **2017**, *18*, 123. [CrossRef] [PubMed]
133. Chakraborty, G.; Jain, S.; Kundu, G.C. Osteopontin Promotes Vascular Endothelial Growth Factor–Dependent Breast Tumor Growth and Angiogenesis via Autocrine and Paracrine Mechanisms. *Cancer Res.* **2008**, *68*, 152–161. [CrossRef] [PubMed]
134. Dai, J.; Peng, L.; Fan, K.; Wang, H.; Wei, R.; Ji, G.; Cai, J.; Lu, B.; Li, B.; Zhang, D.; et al. Osteopontin induces angiogenesis through activation of PI3K/AKT and ERK1/2 in endothelial cells. *Oncogene* **2009**, *28*, 3412–3422. [CrossRef] [PubMed]
135. Raja, R.; Kale, S.; Thorat, D.; Soundararajan, G.; Lohite, K.; Mane, A.; Karnik, S.; Kundu, G.C. Hypoxia-driven osteopontin contributes to breast tumor growth through modulation of HIF1α-mediated VEGF-dependent angiogenesis. *Oncogene* **2014**, *33*, 2053–2064. [CrossRef] [PubMed]
136. Jain, S.; Chakraborty, G.; Kundu, G.C. The Crucial Role of Cyclooxygenase-2 in Osteopontin-Induced Protein Kinase C α/c-Src/IκB Kinase α/β–Dependent Prostate Tumor Progression and Angiogenesis. *Cancer Res.* **2006**, *66*, 6638–6648. [CrossRef]
137. Łukaszewicz-Zając, M.; Pączek, S.; Mroczko, B. A Disintegrin and Metalloproteinase (ADAM) Family—Novel Biomarkers of Selected Gastrointestinal (GI) Malignancies? *Cancers* **2022**, *14*, 2307. [CrossRef]
138. Kobori, T.; Hamasaki, S.; Kitaura, A.; Yamazaki, Y.; Nishinaka, T.; Niwa, A.; Nakao, S.; Wake, H.; Mori, S.; Yoshino, T.; et al. Interleukin-18 Amplifies Macrophage Polarization and Morphological Alteration, Leading to Excessive Angiogenesis. *Front. Immunol.* **2018**, *9*, 334. [CrossRef]
139. Nakagami, H. Cellular senescence and senescence-associated T cells as a potential therapeutic target. *Geriatr. Gerontol. Int.* **2020**, *20*, 97–100. [CrossRef]
140. Ohtani, N. The roles and mechanisms of senescence-associated secretory phenotype (SASP): Can it be controlled by senolysis? *Inflamm. Regen.* **2022**, *42*, 11. [CrossRef]
141. Flanagan, K.C.; Alspach, E.; Pazolli, E.; Parajuli, S.; Ren, Q.; Arthur, L.L.; Tapia, R.; Stewart, S.A. c-Myb and C/EBPβ regulate OPN and other senescence-associated secretory phenotype factors. *Oncotarget* **2018**, *9*, 21–36. [CrossRef]
142. Liu, J.; Xu, K.; Chase, M.; Ji, Y.; Logan, J.K.; Buchsbaum, R.J. Tiam1-regulated osteopontin in senescent fibroblasts contributes to the migration and invasion of associated epithelial cells. *J. Cell Sci.* **2012**, *125*, 376–386. [CrossRef] [PubMed]
143. Xu, K.; Tian, X.; Oh, S.Y.; Movassaghi, M.; Naber, S.P.; Kuperwasser, C.; Buchsbaum, R.J. The fibroblast Tiam1-osteopontin pathway modulates breast cancer invasion and metastasis. *Breast Cancer Res.* **2016**, *18*, 14. [CrossRef] [PubMed]
144. Kyjacova, L.; Saup, R.; Rönsch, K.; Wallbaum, S.; Dukowic-Schulze, S.; Foss, A.; Scherer, S.D.; Rothley, M.; Neeb, A.; Grau, N.; et al. IER2-induced senescence drives melanoma invasion through osteopontin. *Oncogene* **2021**, *40*, 6494–6512. [CrossRef]
145. Zuo, H.; Yang, D.; Wan, Y. Fam20C Regulates Bone Resorption and Breast Cancer Bone Metastasis through Osteopontin and BMP4. *Cancer Res.* **2021**, *81*, 5242–5254. [CrossRef]
146. Kovacheva, M.; Zepp, M.; Schraad, M.; Berger, S.; Berger, M.R. Conditional Knockdown of Osteopontin Inhibits Breast Cancer Skeletal Metastasis. *Int. J. Mol. Sci.* **2019**, *20*, 4918. [CrossRef]
147. Zhao, Y.; Bachelier, R.; Treilleux, I.; Pujuguet, P.; Peyruchaud, O.; Baron, R.; Clément-Lacroix, P.; Clézardin, P. Tumor αvβ3 Integrin Is a Therapeutic Target for Breast Cancer Bone Metastases. *Cancer Res.* **2007**, *67*, 5821–5830. [CrossRef] [PubMed]
148. Terpos, E.; Kiagia, M.; Karapanagiotou, E.M.; Charpidou, A.; Dilana, K.D.; Nasothimiou, E.; Harrington, K.J.; Polyzos, A.; Syrigos, K.N. The clinical significance of serum markers of bone turnover in NSCLC patients: Surveillance, management and prognostic implications. *Anticancer Res.* **2009**, *29*, 1651–1657.
149. Chang, W.-M.; Lin, Y.-F.; Su, C.-Y.; Peng, H.-Y.; Chang, Y.-C.; Hsiao, J.-R.; Chen, C.-L.; Chang, J.-Y.; Shieh, Y.-S.; Hsiao, M.; et al. Parathyroid Hormone-Like Hormone is a Poor Prognosis Marker of Head and Neck Cancer and Promotes Cell Growth via RUNX2 Regulation. *Sci. Rep.* **2017**, *7*, 41131. [CrossRef]
150. Edwards, C.M.; Johnson, R.W. From Good to Bad: The Opposing Effects of PTHrP on Tumor Growth, Dormancy, and Metastasis Throughout Cancer Progression. *Front. Oncol.* **2021**, *11*, 644303. [CrossRef]
151. Pinho, S.S.; Reis, C.A. Glycosylation in cancer: Mechanisms and clinical implications. *Nat. Rev. Cancer* **2015**, *15*, 540–555. [CrossRef]
152. Mereiter, S.; Balmaña, M.; Campos, D.; Gomes, J.; Reis, C.A. Glycosylation in the Era of Cancer-Targeted Therapy: Where Are We Heading? *Cancer Cell* **2019**, *36*, 6–16. [CrossRef] [PubMed]

153. Darling, A.L.; Uversky, V.N. Intrinsic Disorder and Posttranslational Modifications: The Darker Side of the Biological Dark Matter. *Front. Genet.* **2018**, *9*, 158. [CrossRef] [PubMed]
154. Dean, R.A.; Overall, C.M. Proteomics Discovery of Metalloproteinase Substrates in the Cellular Context by iTRAQ™ Labeling Reveals a Diverse MMP-2 Substrate Degradome. *Mol. Cell. Proteom.* **2007**, *6*, 611–623. [CrossRef]
155. Agnihotri, R.; Crawford, H.C.; Haro, H.; Matrisian, L.M.; Havrda, M.C.; Liaw, L. Osteopontin, a Novel Substrate for Matrix Metalloproteinase-3 (Stromelysin-1) and Matrix Metalloproteinase-7 (Matrilysin). *J. Biol. Chem.* **2001**, *276*, 28261–28267. [CrossRef] [PubMed]
156. Yamaguchi, Y.; Shao, Z.; Sharif, S.; Du, X.-Y.; Myles, T.; Merchant, M.; Harsh, G.; Glantz, M.; Recht, L.; Morser, J.; et al. Thrombin-cleaved Fragments of Osteopontin Are Overexpressed in Malignant Glial Tumors and Provide a Molecular Niche with Survival Advantage. *J. Biol. Chem.* **2013**, *288*, 3097–3111. [CrossRef]
157. Takafuji, V.; Forgues, M.; Unsworth, E.; Goldsmith, P.; Wang, X.W. An osteopontin fragment is essential for tumor cell invasion in hepatocellular carcinoma. *Oncogene* **2007**, *26*, 6361–6371. [CrossRef] [PubMed]
158. Mi, Z.; Oliver, T.; Guo, H.; Gao, C.; Kuo, P.C. Thrombin-Cleaved COOH-Terminal Osteopontin Peptide Binds with Cyclophilin C to CD147 in Murine Breast Cancer Cells. *Cancer Res.* **2007**, *67*, 4088–4097. [CrossRef]
159. Yokosaki, Y.; Matsuura, N.; Sasaki, T.; Murakami, I.; Schneider, H.; Higashiyama, S.; Saitoh, Y.; Yamakido, M.; Taooka, Y.; Sheppard, D. The Integrin α9β1 Binds to a Novel Recognition Sequence (SVVYGLR) in the Thrombin-cleaved Amino-terminal Fragment of Osteopontin. *J. Biol. Chem.* **1999**, *274*, 36328–36334. [CrossRef]
160. Senger, D.R.; Perruzzi, C.A.; Papadopoulos-Sergiou, A.; Van de Water, L. Adhesive properties of osteopontin: Regulation by a naturally occurring thrombin-cleavage in close proximity to the GRGDS cell-binding domain. *Mol. Biol. Cell* **1994**, *5*, 565–574. [CrossRef]
161. Yokosaki, Y.; Tanaka, K.; Higashikawa, F.; Yamashita, K.; Eboshida, A. Distinct structural requirements for binding of the integrins alphavbeta6, alphavbeta3, alphavbeta5, alpha5beta1 and alpha9beta1 to osteopontin. *Matrix Biol. J. Int. Soc. Matrix Biol.* **2005**, *24*, 418–427. [CrossRef]
162. Malaponte, G.; Hafsi, S.; Polesel, J.; Castellano, G.; Spessotto, P.; Guarneri, C.; Canevari, S.; Signorelli, S.S.; McCubrey, J.A.; Libra, M. Tumor microenvironment in diffuse large B-cell lymphoma: Matrixmetalloproteinases activation is mediated by osteopontin overexpression. *Biochim. Biophys. Acta* **2016**, *1863*, 483–489. [CrossRef]
163. Lee, M.J.; Yaffe, M.B. Protein Regulation in Signal Transduction. *Cold Spring Harb. Perspect. Biol.* **2016**, *8*, a005918. [CrossRef] [PubMed]
164. Mateos, B.; Holzinger, J.; Conrad-Billroth, C.; Platzer, G.; Żerko, S.; Sealey-Cardona, M.; Anrather, D.; Koźmiński, W.; Konrat, R. Hyperphosphorylation of Human Osteopontin and Its Impact on Structural Dynamics and Molecular Recognition. *Biochemistry* **2021**, *60*, 1347–1355. [CrossRef] [PubMed]
165. Yalak, G.; Vogel, V. Extracellular phosphorylation and phosphorylated proteins: Not just curiosities but physiologically important. *Sci. Signal.* **2012**, *5*, re7. [CrossRef] [PubMed]
166. Yamaguchi, Y.; Hanashima, S.; Yagi, H.; Takahashi, Y.; Sasakawa, H.; Kurimoto, E.; Iguchi, T.; Kon, S.; Uede, T.; Kato, K. NMR characterization of intramolecular interaction of osteopontin, an intrinsically disordered protein with cryptic integrin-binding motifs. *Biochem. Biophys. Res. Commun.* **2010**, *393*, 487–491. [CrossRef] [PubMed]
167. Weber, G.F.; Zawaideh, S.; Hikita, S.; Kumar, V.A.; Cantor, H.; Ashkar, S. Phosphorylation-dependent interaction of osteopontin with its receptors regulates macrophage migration and activation. *J. Leukoc. Biol.* **2002**, *72*, 752–761.
168. Zhang, H.; Cai, Y.-H.; Ding, Y.; Zhang, G.; Liu, Y.; Sun, J.; Yang, Y.; Zhan, Z.; Iliuk, A.; Gu, Z.; et al. Proteomics, Phosphoproteomics and Mirna Analysis of Circulating Extracellular Vesicles through Automated and High-Throughput Isolation. *Cells* **2022**, *11*, 2070. [CrossRef]
169. Tagliabracci, V.S.; Wiley, S.E.; Guo, X.; Kinch, L.N.; Durrant, E.; Wen, J.; Xiao, J.; Cui, J.; Nguyen, K.B.; Engel, J.L.; et al. A Single Kinase Generates the Majority of the Secreted Phosphoproteome. *Cell* **2015**, *161*, 1619–1632. [CrossRef]
170. Liu, X.; Zhan, Y.; Xu, W.; Liu, X.; Geng, Y.; Liu, L.; Da, J.; Wang, J.; Zhang, X.; Jin, H.; et al. Prognostic and immunological role of Fam20C in pan-cancer. *Biosci. Rep.* **2021**, *41*, BSR20201920. [CrossRef]
171. Stowell, S.R.; Ju, T.; Cummings, R.D. Protein Glycosylation in Cancer. *Annu. Rev. Pathol. Mech. Dis.* **2015**, *10*, 473–510. [CrossRef]
172. Kariya, Y.; Oyama, M.; Ohtsuka, M.; Kikuchi, N.; Hashimoto, Y.; Yamamoto, T. Quantitative analysis of β1,6GlcNAc-branched N-glycans on β4 integrin in cutaneous squamous cell carcinoma. *Fukushima J. Med. Sci.* **2020**, *66*, 119–123. [CrossRef] [PubMed]
173. Kariya, Y.; Kariya, Y.; Gu, J. Laminin-332 and Integrins: Signaling Platform for Cell Adhesion and Migration and its Regulation by N-glycosylation. In *Laminins: Structure, Biological Activity and Role in Disease*; Nova Biomedical: New York, NY, USA, 2013; pp. 29–51.
174. Masuda, K.; Takahashi, N.; Tsukamoto, Y.; Honma, H.; Kohri, K. N-Glycan structures of an osteopontin from human bone. *Biochem. Biophys. Res. Commun.* **2000**, *268*, 814–817. [CrossRef] [PubMed]
175. Shanmugam, V.; Chackalaparampil, I.; Kundu, G.C.; Mukherjee, A.B.; Mukherjee, B.B. Altered sialylation of osteopontin prevents its receptor-mediated binding on the surface of oncogenically transformed tsB77 cells. *Biochemistry* **1997**, *36*, 5729–5738. [CrossRef] [PubMed]
176. Patarca, R.; Freeman, G.J.; Singh, R.P.; Wei, F.Y.; Durfee, T.; Blattner, F.; Regnier, D.C.; Kozak, C.A.; Mock, B.A.; Morse, H.C., 3rd; et al. Structural and functional studies of the early T lymphocyte activation 1 (Eta-1) gene. Definition of a novel T cell-dependent response associated with genetic resistance to bacterial infection. *J. Exp. Med.* **1989**, *170*, 145–161. [CrossRef] [PubMed]
177. Klement, J.D.; Poschel, D.B.; Lu, C.; Merting, A.D.; Yang, D.; Redd, P.S.; Liu, K. Osteopontin Blockade Immunotherapy Increases Cytotoxic T Lymphocyte Lytic Activity and Suppresses Colon Tumor Progression. *Cancers* **2021**, *13*, 1006. [CrossRef]

178. Wei, J.; Marisetty, A.; Schrand, B.; Gabrusiewicz, K.; Hashimoto, Y.; Ott, M.; Grami, Z.; Kong, L.-Y.; Ling, X.; Caruso, H.; et al. Osteopontin mediates glioblastoma-associated macrophage infiltration and is a potential therapeutic target. *J. Clin. Investig.* **2019**, *129*, 137–149. [CrossRef]
179. Zhu, Y.; Yang, J.; Xu, D.; Gao, X.-M.; Zhang, Z.; Hsu, J.L.; Li, C.-W.; Lim, S.-O.; Sheng, Y.-Y.; Zhang, Y.; et al. Disruption of tumour-associated macrophage trafficking by the osteopontin-induced colony-stimulating factor-1 signalling sensitises hepatocellular carcinoma to anti-PD-L1 blockade. *Gut* **2019**, *68*, 1653–1666. [CrossRef]
180. Ellert-Miklaszewska, A.; Wisniewski, P.; Kijewska, M.; Gajdanowicz, P.; Pszczolkowska, D.; Przanowski, P.; Dabrowski, M.; Maleszewska, M.; Kaminska, B. Tumour-processed osteopontin and lactadherin drive the protumorigenic reprogramming of microglia and glioma progression. *Oncogene* **2016**, *35*, 6366–6377. [CrossRef]
181. Sangaletti, S.; Tripodo, C.; Sandri, S.; Torselli, I.; Vitali, C.; Ratti, C.; Botti, L.; Burocchi, A.; Porcasi, R.; Tomirotti, A.; et al. Osteopontin Shapes Immunosuppression in the Metastatic Niche. *Cancer Res.* **2014**, *74*, 4706–4719. [CrossRef]
182. Kim, E.-K.; Jeon, I.; Seo, H.; Park, Y.-J.; Song, B.; Lee, K.-A.; Jang, Y.; Chung, Y.; Kang, C.-Y. Tumor-Derived Osteopontin Suppresses Antitumor Immunity by Promoting Extramedullary Myelopoiesis. *Cancer Res.* **2014**, *74*, 6705–6716. [CrossRef]
183. Lin, Y.; Xu, J.; Lan, H. Tumor-associated macrophages in tumor metastasis: Biological roles and clinical therapeutic applications. *J. Hematol. Oncol.* **2019**, *12*, 76. [CrossRef] [PubMed]
184. DeNardo, D.G.; Ruffell, B. Macrophages as regulators of tumour immunity and immunotherapy. *Nat. Rev. Immunol.* **2019**, *19*, 369–382. [CrossRef] [PubMed]
185. Li, X.; Zhang, Q.; Chen, G.; Luo, D. Multi-Omics Analysis Showed the Clinical Value of Gene Signatures of $C1QC^{+}$ and $SPP1^{+}$ TAMs in Cervical Cancer. *Front. Immunol.* **2021**, *12*, 694801. [CrossRef]
186. Qi, J.; Sun, H.; Zhang, Y.; Wang, Z.; Xun, Z.; Li, Z.; Ding, X.; Bao, R.; Hong, L.; Jia, W.; et al. Single-cell and spatial analysis reveal interaction of FAP^{+} fibroblasts and $SPP1^{+}$ macrophages in colorectal cancer. *Nat. Commun.* **2022**, *13*, 1742. [CrossRef] [PubMed]
187. Castello, L.M.; Raineri, D.; Salmi, L.; Clemente, N.; Vaschetto, R.; Quaglia, M.; Garzaro, M.; Gentilli, S.; Navalesi, P.; Cantaluppi, V.; et al. Osteopontin at the Crossroads of Inflammation and Tumor Progression. *Mediat. Inflamm.* **2017**, *2017*, 4049098. [CrossRef]
188. Wykes, M.N.; Lewin, S.R. Immune checkpoint blockade in infectious diseases. *Nat. Rev. Immunol.* **2018**, *18*, 91–104. [CrossRef] [PubMed]
189. Li, Y.; Liu, H.; Zhao, Y.; Yue, D.; Chen, C.; Li, C.; Zhang, Z.; Wang, C. Tumor-associated macrophages (TAMs)-derived osteopontin (OPN) upregulates PD-L1 expression and predicts poor prognosis in non-small cell lung cancer (NSCLC). *Thorac. Cancer* **2021**, *12*, 2698–2709. [CrossRef] [PubMed]
190. Raineri, D.; Dianzani, C.; Cappellano, G.; Maione, F.; Baldanzi, G.; Iacobucci, I.; Clemente, N.; Baldone, G.; Boggio, E.; Gigliotti, C.L.; et al. Osteopontin binds ICOSL promoting tumor metastasis. *Commun. Biol.* **2020**, *3*, 615. [CrossRef]
191. Raineri, D.; Cappellano, G.; Vilardo, B.; Maione, F.; Clemente, N.; Canciani, E.; Boggio, E.; Gigliotti, C.L.; Monge, C.; Dianzani, C.; et al. Inducible T-Cell Costimulator Ligand Plays a Dual Role in Melanoma Metastasis upon Binding to Osteopontin or Inducible T-Cell Costimulator. *Biomedicines* **2021**, *10*, 51. [CrossRef]
192. Louault, K.; Li, R.-R.; DeClerck, Y.A. Cancer-Associated Fibroblasts: Understanding Their Heterogeneity. *Cancers* **2020**, *12*, 3108. [CrossRef]
193. Sahai, E.; Astsaturov, I.; Cukierman, E.; DeNardo, D.G.; Egeblad, M.; Evans, R.M.; Fearon, D.; Greten, F.R.; Hingorani, S.R.; Hunter, T.; et al. A framework for advancing our understanding of cancer-associated fibroblasts. *Nat. Rev. Cancer* **2020**, *20*, 174–186. [CrossRef] [PubMed]
194. Mi, Z.; Bhattacharya, S.D.; Kim, V.M.; Guo, H.; Talbot, L.J.; Kuo, P.C. Osteopontin promotes CCL5-mesenchymal stromal cell-mediated breast cancer metastasis. *Carcinogenesis* **2011**, *32*, 477–487. [CrossRef] [PubMed]
195. Butti, R.; Nimma, R.; Kundu, G.; Bulbule, A.; Kumar, T.V.S.; Gunasekaran, V.P.; Tomar, D.; Kumar, D.; Mane, A.; Gill, S.S.; et al. Tumor-derived osteopontin drives the resident fibroblast to myofibroblast differentiation through Twist1 to promote breast cancer progression. *Oncogene* **2021**, *40*, 2002–2017. [CrossRef]
196. Sharon, Y.; Raz, Y.; Cohen, N.; Ben-Shmuel, A.; Schwartz, H.; Geiger, T.; Erez, N. Tumor-Derived Osteopontin Reprograms Normal Mammary Fibroblasts to Promote Inflammation and Tumor Growth in Breast Cancer. *Cancer Res.* **2015**, *75*, 963–973. [CrossRef] [PubMed]
197. Weber, C.E.; Kothari, A.N.; Wai, P.Y.; Li, N.Y.; Driver, J.; Zapf, M.A.C.; Franzen, C.A.; Gupta, G.N.; Osipo, C.; Zlobin, A.; et al. Osteopontin mediates an MZF1–TGF-β1-dependent transformation of mesenchymal stem cells into cancer-associated fibroblasts in breast cancer. *Oncogene* **2015**, *34*, 4821–4833. [CrossRef] [PubMed]
198. Tokuda, K.; Morine, Y.; Miyazaki, K.; Yamada, S.; Saito, Y.; Nishi, M.; Tokunaga, T.; Ikemoto, T.; Imura, S.; Shimada, M. The interaction between cancer associated fibroblasts and tumor associated macrophages via the osteopontin pathway in the tumor microenvironment of hepatocellular carcinoma. *Oncotarget* **2021**, *12*, 333–343. [CrossRef] [PubMed]
199. Yuan, Q.; Gu, J.; Zhang, J.; Liu, S.; Wang, Q.; Tian, T.; Chen, Z.; Zhang, J. MyD88 in myofibroblasts enhances colitis-associated tumorigenesis via promoting macrophage M2 polarization. *Cell Rep.* **2021**, *34*, 108724. [CrossRef]
200. Pestell, T.G.; Jiao, X.; Kumar, M.; Peck, A.R.; Prisco, M.; Deng, S.; Li, Z.; Ertel, A.; Casimiro, M.C.; Ju, X.; et al. Stromal cyclin D1 promotes heterotypic immune signaling and breast cancer growth. *Oncotarget* **2017**, *8*, 81754–81775. [CrossRef]
201. Lenos, K.J.; Miedema, D.M.; Lodestijn, S.C.; Nijman, L.E.; van den Bosch, T.; Romero Ros, X.; Lourenço, F.C.; Lecca, M.C.; van der Heijden, M.; van Neerven, S.M.; et al. Stem cell functionality is microenvironmentally defined during tumour expansion and therapy response in colon cancer. *Nat. Cell Biol.* **2018**, *20*, 1193–1202. [CrossRef]
202. Qin, X.; Yan, M.; Wang, X.; Xu, Q.; Wang, X.; Zhu, X.; Shi, J.; Li, Z.; Zhang, J.; Chen, W. Cancer-associated Fibroblast-derived IL-6 Promotes Head and Neck Cancer Progression via the Osteopontin-NF-kappa B Signaling Pathway. *Theranostics* **2018**, *8*, 921–940. [CrossRef]

203. Jing, C.-Y.; Fu, Y.-P.; Zhou, C.; Zhang, M.-X.; Yi, Y.; Huang, J.-L.; Gan, W.; Zhang, J.; Zheng, S.-S.; Zhang, B.-H.; et al. Hepatic stellate cells promote intrahepatic cholangiocarcinoma progression via NR4A2/osteopontin/Wnt signaling axis. *Oncogene* **2021**, *40*, 2910–2922. [CrossRef] [PubMed]
204. Nazarizadeh, A.; Alizadeh-Fanalou, S.; Hosseini, A.; Mirzaei, A.; Salimi, V.; Keshipour, H.; Safizadeh, B.; Jamshidi, K.; Bahrabadi, M.; Tavakoli-Yaraki, M. Evaluation of local and circulating osteopontin in malignant and benign primary bone tumors. *J. Bone Oncol.* **2021**, *29*, 100377. [CrossRef] [PubMed]
205. Ji, X.; Liu, Y.; Mei, F.; Li, X.; Zhang, M.; Yao, B.; Wu, R.; You, J.; Pei, F. SPP1 overexpression is associated with poor outcomes in ALK fusion lung cancer patients without receiving targeted therapy. *Sci. Rep.* **2021**, *11*, 14031. [CrossRef] [PubMed]
206. Moldogazieva, N.; Mokhosoev, I.; Zavadskiy, S.; Terentiev, A. Proteomic Profiling and Artificial Intelligence for Hepatocellular Carcinoma Translational Medicine. *Biomedicines* **2021**, *9*, 159. [CrossRef]
207. Anborgh, P.H.; Caria, L.B.; Chambers, A.F.; Tuck, A.B.; Stitt, L.W.; Brackstone, M. Role of plasma osteopontin as a biomarker in locally advanced breast cancer. *Am. J. Transl. Res.* **2015**, *7*, 723–732. [PubMed]
208. Kohata, T.; Ito, S.; Masuda, T.; Furuta, T.; Nakada, M.; Ohtsuki, S. Laminin Subunit Alpha-4 and Osteopontin Are Glioblastoma-Selective Secreted Proteins That Are Increased in the Cerebrospinal Fluid of Glioblastoma Patients. *J. Proteome Res.* **2020**, *19*, 3542–3553. [CrossRef] [PubMed]
209. Shang, S.; Plymoth, A.; Ge, S.; Feng, Z.; Rosen, H.R.; Sangrajrang, S.; Hainaut, P.; Marrero, J.A.; Beretta, L. Identification of osteopontin as a novel marker for early hepatocellular carcinoma. *Hepatology* **2012**, *55*, 483–490. [CrossRef]
210. Sun, T.; Tang, Y.; Sun, D.; Bu, Q.; Li, P. Osteopontin versus alpha-fetoprotein as a diagnostic marker for hepatocellular carcinoma: A meta-analysis. *Oncotargets Ther.* **2018**, *11*, 8925–8935. [CrossRef]
211. Sun, J.; Chen, X.; Wang, Y. Comparison of the diagnostic value of CEA combined with OPN or DKK1 in non-small cell lung cancer. *Oncol. Lett.* **2020**, *20*, 3046–3052. [CrossRef]
212. Walker, C.; Nguyen, T.-M.; Jessel, S.; Alvero, A.B.; Silasi, D.-A.; Rutherford, T.; Draghici, S.; Mor, G. Automated Assay of a Four-Protein Biomarker Panel for Improved Detection of Ovarian Cancer. *Cancers* **2021**, *13*, 325. [CrossRef]
213. Hasenburg, A.; Eichkorn, D.; Vosshagen, F.; Obermayr, E.; Geroldinger, A.; Zeillinger, R.; Bossart, M. Biomarker-based early detection of epithelial ovarian cancer based on a five-protein signature in patient's plasma—A prospective trial. *BMC Cancer* **2021**, *21*, 1037. [CrossRef] [PubMed]
214. Chen, Y.; Liu, H.; Wu, W.; Li, Y.; Li, J. Osteopontin genetic variants are associated with overall survival in advanced non-small-cell lung cancer patients and bone metastasis. *J. Exp. Clin. Cancer Res.* **2013**, *32*, 45. [CrossRef] [PubMed]
215. Miao, T.w.; Xiao, W.; Du, L.y.; Mao, B.; Huang, W.; Chen, X.m.; Li, C.; Wang, Y.; Fu, J.j. High expression of SPP1 in patients with chronic obstructive pulmonary disease (COPD) is correlated with increased risk of lung cancer. *FEBS Open Bio* **2021**, *11*, 1237–1249. [CrossRef] [PubMed]
216. Zhang, X.; Bi, K.; Tu, X.; Zhang, Q.; Cao, Q.; Liang, Y.; Zeng, P.; Wang, L.; Liu, T.; Fang, W.; et al. Interleukin-33 as an early predictor of cetuximab treatment efficacy in patients with colorectal cancer. *Cancer Med.* **2021**, *10*, 8338–8351. [CrossRef]
217. Armstrong, A.J.; Nixon, A.B.; Carmack, A.; Yang, Q.; Eisen, T.; Stadler, W.M.; Jones, R.J.; Garcia, J.A.; Vaishampayan, U.N.; Picus, J.; et al. Angiokines Associated with Targeted Therapy Outcomes in Patients with Non–Clear Cell Renal Cell Carcinoma. *Clin. Cancer Res.* **2021**, *27*, 3317–3328. [CrossRef]
218. Carbone, F.; Grossi, F.; Bonaventura, A.; Vecchié, A.; Minetti, S.; Bardi, N.; Elia, E.; Ansaldo, A.M.; Ferrara, D.; Rijavec, E.; et al. Baseline serum levels of osteopontin predict clinical response to treatment with nivolumab in patients with non-small cell lung cancer. *Clin. Exp. Metastasis* **2019**, *36*, 449–456. [CrossRef]
219. Sperlich, A.; Balmert, A.; Doll, D.; Bauer, S.; Franke, F.; Keller, G.; Wilhelm, D.; Mur, A.; Respondek, M.; Friess, H.; et al. Genetic and immunological biomarkers predict metastatic disease recurrence in stage III colon cancer. *BMC Cancer* **2018**, *18*, 998. [CrossRef]
220. Shojaei, F.; Scott, N.; Kang, X.; Lappin, P.B.; Fitzgerald, A.A.; Karlicek, S.; Simmons, B.H.; Wu, A.; Lee, J.H.; Bergqvist, S.; et al. Osteopontin induces growth of metastatic tumors in a preclinical model of non-small lung cancer. *J. Exp. Clin. Cancer Res.* **2012**, *31*, 26. [CrossRef]
221. Dai, J.; Li, B.; Shi, J.; Peng, L.; Zhang, D.; Qian, W.; Hou, S.; Zhao, L.; Gao, J.; Cao, Z.; et al. A humanized anti-osteopontin antibody inhibits breast cancer growth and metastasis in vivo. *Cancer Immunol. Immunother.* **2010**, *59*, 355–366. [CrossRef]
222. Boumans, M.J.; Houbiers, J.G.; Verschueren, P.; Ishikura, H.; Westhovens, R.; Brouwer, E.; Rojkovich, B.; Kelly, S.; den Adel, M.; Isaacs, J.; et al. Safety, tolerability, pharmacokinetics, pharmacodynamics and efficacy of the monoclonal antibody ASK8007 blocking osteopontin in patients with rheumatoid arthritis: A randomised, placebo controlled, proof-of-concept study. *Ann. Rheum. Dis.* **2012**, *71*, 180–185. [CrossRef]
223. Farrokhi, V.; Chabot, J.R.; Neubert, H.; Yang, Z. Assessing the Feasibility of Neutralizing Osteopontin with Various Therapeutic Antibody Modalities. *Sci. Rep.* **2018**, *8*, 7781. [CrossRef] [PubMed]
224. Bergonzini, C.; Kroese, K.; Zweemer, A.J.M.; Danen, E.H.J. Targeting Integrins for Cancer Therapy—Disappointments and Opportunities. *Front. Cell Dev. Biol.* **2022**, *10*, 863850. [CrossRef]
225. Slack, R.J.; Macdonald, S.J.F.; Roper, J.A.; Jenkins, R.G.; Hatley, R.J.D. Emerging therapeutic opportunities for integrin inhibitors. *Nat. Rev. Drug Discov.* **2022**, *21*, 60–78. [CrossRef] [PubMed]
226. Ben-David-Naim, M.; Dagan, A.; Grad, E.; Aizik, G.; Nordling-David, M.; Morss Clyne, A.; Granot, Z.; Golomb, G. Targeted siRNA Nanoparticles for Mammary Carcinoma Therapy. *Cancers* **2019**, *11*, 442. [CrossRef] [PubMed]
227. Noguchi-Yachide, T. BET Bromodomain as a Target of Epigenetic Therapy. *Chem. Pharm. Bull.* **2016**, *64*, 540–547. [CrossRef]

228. Deng, G.; Zeng, F.; Su, J.; Zhao, S.; Hu, R.; Zhu, W.; Hu, S.; Chen, X.; Yin, M. BET inhibitor suppresses melanoma progression via the noncanonical NF-κB/SPP1 pathway. *Theranostics* **2020**, *10*, 11428–11443. [CrossRef]
229. Yamanaka, T.; Harimoto, N.; Yokobori, T.; Muranushi, R.; Hoshino, K.; Hagiwara, K.; Gantumur, D.; Handa, T.; Ishii, N.; Tsukagoshi, M.; et al. Conophylline Inhibits Hepatocellular Carcinoma by Inhibiting Activated Cancer-associated Fibroblasts Through Suppression of G Protein–coupled Receptor 68. *Mol. Cancer Ther.* **2021**, *20*, 1019–1028. [CrossRef]
230. Benedicto, A.; Hernandez-Unzueta, I.; Sanz, E.; Márquez, J. Ocoxin Increases the Antitumor Effect of BRAF Inhibition and Reduces Cancer Associated Fibroblast-Mediated Chemoresistance and Protumoral Activity in Metastatic Melanoma. *Nutrients* **2021**, *13*, 686. [CrossRef]
231. Chiou, J.; Chang, Y.-C.; Tsai, H.-F.; Lin, Y.-F.; Huang, M.-S.; Yang, C.-J.; Hsiao, M. Follistatin-like Protein 1 Inhibits Lung Cancer Metastasis by Preventing Proteolytic Activation of Osteopontin. *Cancer Res.* **2019**, *79*, 6113–6125. [CrossRef]
232. Marin-Acevedo, J.A.; Kimbrough, E.O.; Lou, Y. Next generation of immune checkpoint inhibitors and beyond. *J. Hematol. Oncol.* **2021**, *14*, 45. [CrossRef]
233. Li, Q.; Wang, Y.; Jia, W.; Deng, H.; Li, G.; Deng, W.; Chen, J.; Kim, B.Y.S.; Jiang, W.; Liu, Q.; et al. Low-Dose Anti-Angiogenic Therapy Sensitizes Breast Cancer to PD-1 Blockade. *Clin. Cancer Res.* **2020**, *26*, 1712–1724. [CrossRef] [PubMed]
234. Xu, M.; Mao, C.; Chen, H.; Liu, L.; Wang, Y.; Hussain, A.; Li, S.; Zhang, X.; Tuguntaev, R.G.; Liang, X.J.; et al. Osteopontin targeted theranostic nanoprobes for laser-induced synergistic regression of vulnerable atherosclerotic plaques. *Acta Pharm. Sin. B* **2022**, *12*, 2014–2028. [CrossRef] [PubMed]