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Osteopontin as a target for cancer therapy

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1. ABSTRACT

Osteopontin (OPN) is a glycophosphoprotein cytokine that has multiple functions. OPN is expressed and secreted by various cells, and has a role in cell adhesion, chemotaxis, prevention of apoptosis, invasion, migration and anchorage-independent growth of tumor cells. Extensive research has demonstrated the pivotal participation of OPN in the regulation of cell signaling which controls neoplastic and malignant transformation. The elevated expression of OPN has been observed in a variety of cancers. OPN has been linked with tumor metastasis and signifies a poor prognosis for the patient. This review details the mechanisms by which OPN facilitates these pathological events. It will also show that gaining an understanding of the mechanism of OPN's action at a cellular level has led to the development of a number of therapeutic strategies against the cytokine. These include inhibiting its expression, antagonizing cell surface receptor activation and blocking downstream cell signaling pathways. In addition to the potential of these therapies, serum levels of OPN could be used as a diagnostic and prognostic marker. The authors propose that with further research and development, osteopontin directed treatment could greatly enhance outcomes for cancer patients.

2. INTRODUCTION

Cancer cells undertake limitless replication whilst evading mechanisms causing cell death. Control of cell signaling pathways go awry, resulting in the usurping of normal control processes to produce what was termed "the hallmarks of cancer" by Hanahan and Weinberg in 2000 (1). These hallmarks are biological traits that are widely shared by solid tumor cells that serve to perpetuate the growth of the cancer. These traits are defined as self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, tissue invasion and metastasis, sustained angiogenesis, and limitless potential for replication. Clinically, metastatic tumors are difficult to treat, underlining the malignancy of tumors that are able to spread from the site of primary growth. It is the metastatic phenotype of the tumor cells that makes them refractive to treatment (2, 3) resulting in a poor prognosis (4-6). A better understanding of the signaling pathways that lead to metastasis would help to develop treatments that could control the spread of the primary tumor.

Tumorigenesis and metastasis involve a sequence of complex cellular functions. Metastasis occurs when the tumor cells acquire the ability to disseminate and spread. The cells must degrade extra-cellular proteins, engage in cell-to-cell adhesion, be motile, evade immunological destruction, avoid induction of tumor cell apoptosis, and initiate angiogenesis. An effective therapy for cancer will inhibit tumorigenesis and/or metastasis. New treatments will be based on novel identified targets that are involved in the induction of these processes. Research for the past decade has resulted in finding many such molecules that have a role in mediating metastasis. One such key molecule is osteopontin, which has been shown as pivotal in mediating metastasis in many types of cancer (7).

This review will describe how osteopontin (OPN) contributes to development of the malignant phenotype. It will become clear that OPN is essential to this development, and as such has potential as a broad-spectrum target for anti-cancer therapy. The role OPN plays in specific cancers such as breast, lung, brain and colon will be considered, as well as the potential for the protein to be used as a diagnostic or prognostic marker to denote the onset of metastatic disease (8-10). Various strategies that could be used to target OPN-dependant mechanisms will be described.

3. OSTEOPONTIN

3.1. Osteopontin gene structure

Osteopontin is a significant constituent of bone, and also has been detected in cartilage, brain, kidney, activated macrophages, lymphocytes, and vascular smooth muscle cells. It is secreted by osteoclasts, osteoblasts and osteocytes (11, 12); these various sites indicate the different cellular contexts in which osteopontin functions.

A single copy of the human osteopontin gene is found on the long arm of chromosome 4 (4q21-4q25) (11) spanning approximately 11 kilobases, 6 of the 7 exons in the gene contain protein-coding sequence (13).

3.2. Regulation of osteopontin transcription

Osteopontin expression can be up-regulated by different factors affecting different cell types (reviewed in (11)) including hypoxia (14, 15), Vitamin D3 (16), estrogen (17), retinoic acid (18), PDGF, EGF, TGF-β, and bone morphogenic proteins (reviewed in (19)). Cells treated with TPA, dexamethasone and conavalin A all will increase osteopontin expression (11).

The small GTP-binding protein *ras* is instrumental in the activation of signaling pathways - including the mitogen-activated protein kinase (MAPK) and the PI3K/Akt pathways - leading to osteopontin expression (20). The Rous sarcoma oncogene Src regulates osteopontin levels (21). Constitutive activation of the Wnt signaling pathway results in deregulation of OPN expression (22). BCR-ABL oncogene has been reported to up-regulate osteopontin expression via a Raf-1 and MEK-1/2 dependent pathway (23, 24).

Transcriptional regulation is not fully defined, but many potential regulatory sequences are reported in the region upstream of the gene. TATA-like and CCAAT-like sequences have been found, along with vitamin-D responsive-like motifs, and GATA-1, AP-1, SP-1, USF and Ets transcription factors, including Ets-1, Ets-2, PEA-3, ERM and Runx binding sequences (25).

Functional studies have defined a role for factors in up-regulating osteopontin gene transcription in normal and cancerous cells. Those reported as having a role include ETS-1, ETS-2, PEA-3, Runx, TCF-1, β-catenin/LEF-1, c-jun, USF, AP-1, p53, RE-1, ERα, Vitamin D receptor, SP1 (reviewed in (7), and AP-2alpha (26).

Significantly, down-regulators of transcription have also been reported. Wild type BRCA-1 binds estrogen receptor α, AP-1 and PEA3 and inhibits OPN mRNA levels, while mutant BRCA-1 impedes this suppression (27). Also, the transcription factor TCF-4 was shown to inhibit initiation of OPN transcription (28).

3.3. Osteopontin protein structure

Senger *et al* (1979) described OPN as a "transformation-associated phosphoprotein" (29). The protein has been given different names such as 44-kD bone phosphoprotein, 2ar, Eta-1 (early T lymphocyte activation-1) and 2B7, each name corresponding to a site of secretion/discovery and shows that OPN can be found in various different tissues and cell types (12). This serves to introduce the concept that OPN is a multi-functioning protein within normal cell physiology. The protein is now exclusively referred to as osteopontin; this name arose from its discovery in bone cells (7).

Human OPN protein contains 314 amino acids with a predicted molecular mass of 32 kDa. Due to extensive post-translational modification, involving phosphorylation, glycosylation and sulphation, the observed molecular weight by gel electrophoresis is species dependent in a range of 30-75 kDa. It has been proposed that these modifications provide functional variability of

the molecule in different cellular contexts (11). The molecule appears to largely lack organized secondary structure. Across various species there is a 40% consensus in gene sequences; this consensus is reflected in conserved amino acid motifs at the protein level (7). The conserved sequences include a GRGDS (glycine-arginine-glycine-aspartate-serine) domain, more commonly referred to as the RGD domain, a thrombin cleavage site and an aspartate-rich region. As these domains and regions are conserved in all species it is assumed that they are essential for OPN to carry out its cellular biological functions.

4. CELL SURFACE RECEPTORS OF OSTEOPONTIN

Osteopontin must be secreted from cells in order for it to produce a cellular effect. Osteopontin actions, whether physiological or pathological, are mediated through interaction with cell surface receptors. The main receptors for OPN belong to the integrin and CD44 receptor families.

4.1. The integrin family of receptors

This family of cell surface receptors mediates a variety of cell-to-cell and cell-to-matrix interactions. Integrins are transmembrane dimeric proteins consisting of α and β subunits (30). Each heterodimer can bind to a number of different ligands including fibronectin, vitronectin and OPN (31). Osteopontin has been included in the SIBLING (*s*mall *i*ntegrin-*b*inding *l*igand, *N*-linked glycoproteins) family of proteins (32). Members of this family share a number of common characteristics including a common genetic locus on human chromosome 4, and an RGD domain. The RGD domain has been shown to be a site of interaction with integrins (33-35). Human OPN has two integrin-binding domains, the typical RGD domain, which interacts with $\alpha_v\beta_3$ integrin, and the atypical SVVYGLR domain, which is the site of $\alpha_9\beta_1$ integrin binding (36). It is thought that this second integrin-binding domain may be calcium-dependant and its significance remains unclear.

4.1.1. Osteopontin/intergrin binding

Of the 24 αβ-heterodimers currently documented, OPN interacts with $\alpha_4\beta_1$, $\alpha_5\beta_1$, $\alpha_8\beta_1$, $\alpha_9\beta_1$, $\alpha_v\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_v\beta_6$, and $\alpha_4\beta_7$ integrins (30). The integrin binds to either the RGD or the SVVYGLR motif (which is revealed after thrombin cleavage of osteopontin) (37).

Integrin activation leads to signal transduction through several pathways: via the Rous sarcoma oncogene Src to either EGFR/MAPK or PI3K/Akt; NF-kappaB (38); and MEKK to JNK1. Another important pathway is activated by the interaction between the thrombin-cleaved OPN derivative and the $\alpha_v\beta_3$ integrin. This interaction leads to phosphorylation of focal adhesion kinase (FAK), paxillin, tensin and Src, which in turn initiate downstream signals (25).

4.2. The CD44 family of receptors

CD44, a cell surface glycoprotein also known as the hyaluronan receptor (39) has multiple functions and binding partners (40). The protein consists of a C-terminal cytoplasmic domain, a transmembrane domain and an extra-cellular domain. Many isoforms of the protein exist all originating from the same gene. The structural and functional differences of the isoforms can be attributed to the various splicing and post-translational modifications that occur. The most common isoform of the protein is CD44s, which contains 363 amino acids and is the variant that engages with OPN through a peptide interaction (41). The main ligand that binds to CD44 is the extracellular matrix (ECM) component hyaluronic acid (HA); OPN/CD44 binding is inhibited by both HA and anti-CD44 antibodies, demonstrating the interaction between OPN and receptor (42).

Binding of OPN with αvβ3 integrin can upregulate the expression of CD44v6. There is evidence to suggest that CD44 variant CD44v6 and the β1-containing integrins bound to OPN will cooperate to promote downstream functions (43).

5. OSTEOPONTIN FUNCTION

5.1. Cell migration and adhesion

Osteopontin acts as an adhesion molecule and chemo-attractant to many cell types; monocytes/macrophages, T cells, smooth muscle cells, endothelial cells, epithelial cells, and several malignant cancer cell types will migrate towards a source of the protein (38, 44-49)

5.2. Tissue remodelling and wound healing

Signal transduction after OPN activation of cell surface receptors results in the increased expression of a number of proteolytic enzymes capable of degrading extracellular matrix proteins. These enzymes include matrix metallo proteinases (MMPs) and urokinase plasminogen activator (uPA) (50). In this way OPN mediates the changes necessary to facilitate 3-dimensional changes at the site of developing tissue or injury (51).

OPN knockout mice display defective repair mechanisms characterized by abnormal collagen fibrillogenesis upon incisional skin wounds (52). Osteopontin expression was shown to be concordant with regenerating tubules in rat models after acute renal failure induced by the nephrotoxic drug gentamicin (53). OPN's remodelling function was attributed to be responsible for the proliferation of vascular smooth muscle cells and glomerular mesangial cells following hypoxia (54, 55).

5.3. Bone homeostasis

Bone integrity is maintained by a balance of the opposing actions of cells within the tissue. Removal of bone is carried out by osteoclasts, whilst new bone is laid down by osteoblasts (38). Osteopontin acts within bone homeostasis in three ways. It inhibits the mineralisation process by binding to hydroxyapatite, promotes differentiation of precursor cells to become osteoclasts, and enhances the activity of osteoblasts (11, 19, 38).

5.4. INFLAMMATION

Osteopontin also plays a role in the negotiation of cell injury and infection, again with apparently opposing functions. Upon injury, OPN acts in a pro-inflammatory fashion, attracting macrophages and T cells, and accelerating early acute immune responses. After the initial reaction to injury has been mounted however, OPN functions in an anti-inflammatory role, to contain the extent of the injury and enhance wound healing, by inhibiting inducible nitric oxide synthase (iNOS), inhibiting enzymes responsible for tissue remodeling, and by shifting the nature of the immune response from the innate cell-mediated mode to the adaptive humoral system (51).

5.5. Cell survival and proliferation

OPN acts in a protective/proliferative manner in different cells depending upon cellular context. Epithelial cells will proliferate upon treatment with OPN (56), and endothelial cells adhering to OPN-coated surfaces are protected against induced apoptosis (38); this has been shown to be mediated by osteopontin binding to αvβ3, in turn activating pro-survival transcriptional factor NF kappa B (57). Increased tubular cell apoptosis in obstructed kidneys of OPN knockout mice highlights/underscores the importance of OPN in cell survival (58). Also, cells grown in suspension can be protected by osteopontin from stress-induced apoptosis (38).

6. OSTEOPONTIN FUNCTION IN CANCER

The correlation of the expression of osteopontin by cancer cells with the ability to metastasize has been well documented (8, 59-61), along with observations that inhibition of OPN expression will reduce the metastatic potential of previously more malignant cells (62). This evidence leads the authors to suggest that the appropriation of the normal physiological functions of osteopontin by tumor cells enables them to 'progress' i.e. achieve a metastatic phenotype. Osteopontin participation in tumor progression may simply be an unfortunate but vital consequence of the events leading to transformation. For instance, when NIH3T3 cells are transformed with either T24 H-ras or H-RasV12 (constitutively activated *ras* mutants), increased OPN expression results, and the cells display a more malignant phenotype (63, 64). *Ras* is a commonly mutated gene in many types of cancer (62, 65).

Tumor progression driven by osteopontin is accompanied by a concomitant change in the expression of the cell surface receptors. The $\alpha_v\beta_3$ integrin receptor is found to be over-expressed in many tumor cells (7, 66) and is associated with increasing metastatic ability of cancer cells (25, 67). There are also reports that an increase in CD44 expression results in a more aggressive tumor, and an increase in the metastatic capability of the tumor cells through its interaction with OPN (39). This interaction is thought to selectively induce CD44-dependant chemotaxis, which aids in the migration and invasion of tumor cells (9).

By considering the "hallmarks of cancer", it is possible to define how cells have gained function through osteopontin to achieve characteristics beyond invasion and metastasis. It can be seen just how pivotal the role of osteopontin in malignant disease is (68).

6.1. Self-sufficiency in growth signals

Tumor cells display an independence from external proliferation signals. This is achieved in different ways: autocrine stimulation, cell surface receptor deregulation, or signaling pathway activation (1). Osteopontin can enhance growth factor-induced cell proliferation in different cancer cells, and may act as an autocrine growth factor itself (7). Osteopontin from other sources (for example, activated macrophages) can also serve to supplement growth factors expressed as a result of transformation (69).

6.2. Evasion of apoptosis

The loss of cells through programmed cell death is another obstacle that is overcome by cancer cells. Denhardt *et al* (2001) propose that osteopontin can produce an anti-apoptotic signal (51). For instance, in a gastric cancer cell line, treatment with osteopontin results in a resistance to UV-induced apoptosis (70). Osteopontin can also protect cells from apoptosis induced as a result of detachment of cells: their growth becomes anchorage independent (71).

6.3. Sustained angiogenesis

In order for tumors to grow, cells require oxygen and nutrients. Capillary vessels are required to allow the expansion of the tumor and provide at least a notional route for further metastasis. In some cell lines, hypoxia induces increased osteopontin expression (72-74) as well as more recognised angiogenic factors (75). Experiments where osteopontin is forcibly over-expressed in cancer cell lines, which are then used in animals, display increased angiogenesis (76). Osteopontin increases endothelial cell migration (77), and can co-operate with VEGF to enhance blood vessel formation, or induce angiogenesis independently of VEGF (50).

6.4. Tissue invasion and metastasis

The movement of cells away from the primary growth to establish distal sites where space and/or nutrients cannot limit growth is the characteristic of malignant disease that makes it most difficult to successfully treat (2, 3). Osteopontin is predominantly associated with cancer in relation to metastasis, and has an important role in all phases of the process.

Malignant cancer cells show an increased sensitivity to OPN, rendering them more motile (38). Consistent with its function as a chemoattractant, in some types of cancer OPN has been characterised as a homing molecule, facilitating the increased frequency of bone metastases (78).

Osteopontin also controls expression and secretion of MMPs and uPA, enzymes that are essential for the breakdown of basement membrane and the extracellular matrix (37). Digestion of supporting tissues surrounding the primary tumor, coupled with increased motility of the tumor cells, facilitates metastasis.

7. OSTEOPONTIN AND CLINICAL CANCER – EXPRESSION IN TUMORS

Experimental work has established that over-expression of OPN yields cells in culture with greater potential to produce tumors and metastases (8). This over-expression is seen in samples from tumors taken in a clinical context: further, a correlation between level of expression and the staging of the tumor has been reported (59, 79).

Work linking osteopontin expression to transformation and metastasis is proceeding in many different cancer types; however the majority of the research has been carried out in breast, prostate, lung, colorectal, and brain cancer.

The source of the osteopontin has been examined in different studies. Brown *et al* (1994) conducted an extensive study to check for the OPN expression and distribution in 11 different human carcinomas. Osteopontin mRNA was found to be higher in the cancer tissue compared to the corresponding normal tissues. Interestingly the high mRNA levels was observed in macrophages associated with tumor cells and not in the tumor cells, whereas OPN protein was found to be high in both tumor cells and macrophages, suggesting that macrophage secreted OPN was bound to proximal tumor cells (80) .

Rittling *et al* (1997) studied the expression pattern of OPN in mouse mammary glands during different stages of post-natal development. They showed that the levels of OPN expression remained low to moderate in virgin and pregnant mice, whereas there was an up regulation of osteopontin during initial days of lactation which sustained through 9 days of involution (81). In breast tumors, OPN plays a functional role in altering the cancer cells in such a way that allows for tumor progression and metastasis to occur (68). He *et al* (2006) identified functionally different splice variants of OPN expressed by a breast cancer cell line. Of particular interest is the splice variant OPN-c which aids cell migration by inducing cell survival in non-adherent conditions. OPN-c was found to be specifically expressed by transformed cells and was not detected in surrounding normal tissues (71). Cell invasion occurs by degradation of the basement membrane and this process is dependent upon the proteolytic activity generated by MMPs and the plasminogen-activator–plasmin system. Studies by Fisher *et al* (2000) have suggested that the interaction between OPN and uPA results in increased cell invasion (82). Using a combination of gene expression and tissue microarrays, Irby *et al* (2004) showed that increased OPN expression is concordant with tumor stage in colon cancer (83). They have shown that OPN enhances motility and invasive capacity of human colon cancer cells *in vitro*. stable transfection of low tumorigenic human colon cancer cell lines with OPN also resulted in enhanced tumorigenicity *in vivo*. It was suggested that OPN appeared to regulate motility though interaction with CD44. Rohde *et al* (2007) conducted a transcriptome study on colorectal cancer and identified the gene OPN among the most strongly up-regulated transcript when compared with precancerous cells and early stage disease cells (22). They analyzed 13 normal colon tissues, 9 adenomas, 120 primary colon tumors, and 10 liver metastases by quantitative reverse-transcription PCR. OPN expression was strongly elevated in primary colon cancer and liver metastasis, but not in pre-cancerous lesions and UICC stages I tumors underlining the importance of osteopontin in metastatic process in these tumors. They elucidated that OPN as a transcriptional target of aberrant *Wnt* signaling and have shown the same effect in genetically defined mouse models.

8. OSTEOPONTIN AS A TOOL IN CLINICAL CANCER THERAPY

8.1. OPN as a prognostic marker

The expression of OPN occurs normally within the body (11), but the levels expressed in tumors have been found to be elevated. Increased expression is associated with aggressive tumor progression, metastasis and poor patient prognosis (22, 59, 79, 84).

Blood levels of osteopontin have been identified as a marker for cancer progression and prognosis in different types of cancer (for example head and neck (85) and hepatocellular cancers (86)), but their potential goes beyond that of prognosis because of their fundamental participation in the cellular changes of invasion and metastasis. Inhibition of osteopontin, or downstream inactivation would inhibit, at least in part, the progression of a tumor and prevent spread to distal sites. Tuck and Chambers found that negative patient prognosis (decreased disease-free state and increased mortality) was correlated with high levels of OPN within the breast tumors (87). Bramwell *et al* (2006) found that OPN levels in blood could be used as a reliable predictive prognostic marker in patients with metastatic breast cancer (88). As with tumor cells, high levels of OPN in the plasma were indicative of an aggressive tumor and poor patient prognosis for those with metastatic breast cancer.

OPN mRNA as well as protein levels were detected at higher levels in malignant glioblastomsa with respect to non-neoplastic and benign astrocytomas (89). Elevated OPN levels are reported in plasma and cerebrospinal fluid of patients with atypical tertoid/rhabdoid tumors (90).

OPN has been identified as a potential biomarker for ovarian cancer in a study using a cDNA microarray (91). In another study, OPN levels were measured using enzyme-linked immunosorbent assay (ELISA) in preoperative plasma from patients with epithelial ovarian cancer and healthy controls. OPN levels were significantly higher in women with ovarian cancer compared to the controls and also with respect to other types of gynaecological cancers such as cervical and endometrial cancer (92).

Zhou *et al* (2005) conducted a study comparing metastatic tumor nodules with benign skin lesions using DNA microarrays and immunohistochemical staining (93).

OPN was found to be one among 189 other genes that were implicated in metastatic transition. OPN treated B16 melanoma cells showed a significant increase in tumor burden in mouse models (94).

From these studies it should be clear that both tumor cell and plasma OPN have potential to be prognostic markers in determining the level of malignancy.

8.2. Inhibition of osteopontin

8.2.1. Suppression of the osteopontin message

There are currently several strategies to inhibit gene expression at the mRNA level, which include ribozyme cleavage, antisense oligonucleotides and siRNA. In these techniques, mRNA is degraded and the protein not expressed. However, these strategies are difficult to deliver into tissues. Small RNA endonucleases (ribozymes) reversibly cleave the phosphodiester bond of substrate RNA to generate 5′-hydroxyl and 2′,3′-cyclic phosphate termini, therefore specifically inhibiting the expression of target genes. Three hammerhead ribozymes designed to cleave three different regions of osteopontin mRNA reduced osteopontin expression in transformed cells (95). It has been also shown that expression of anti-sense osteopontin RNA in metastatic ras-transformed NIH3T3 mouse fibroblasts is associated with reduced malignancy. Anti-sense inhibition of osteopontin expression may suffice to prevent full transformation. Stable transfection of osteopontin anti-sense RNA in highly metastatic Rama 37-Met-DNA cells prevented increased osteopontin expression at both mRNA and protein levels, and cells failed to form colonies in soft agar medium and metastasize *in vivo* (27). However, Cheng *et al* (2007) demonstrated that macrophage-derived OPN restored the metastatic potential of cells lacking OPN, and recommend that cancer cell-stroma cell interactions should be taken into account when designing anti-cancer treatments (69).

8.2.2. Inhibition of osteopontin signaling

Inhibition of protein activity is most frequently attempted using antibodies, or synthetic peptides. Polyclonal antibodies to osteopontin inhibit the growth stimulatory effect of endogenous osteopontin in human prostate carcinoma cells and inhibited the adhesion of MDA-MB-435 breast cancer cells (96). Cell adhesion is mediated by binding of the GRGDS domain in osteopontin to integrin receptors on tumor cells. Synthetic peptides containing the RGD domain are regularly used to block integrin binding with osteopontin (97).

8.2.3. Modulation of post-translational modifications

The extensive regulation of osteopontin function by post-transcriptional modification and the inferred structural differences between tumor-derived and T-cell-secreted osteopontin may imply that their targeting by therapeutics would be desirable. Thrombin cleavage of osteopontin is a prerequisite for activation of its integrin-binding domain. Different thrombin inhibitors are in clinical trials (98). Osteopontin is a substrate for casein kinase (99) and for tartrate-resistant acid phosphatase. The phosphorylation status of osteopontin upon secretion is influenced by its mode of induction. 1α,25-Dihydroxy-vitamin D_3 and estrogens can both affect the extent of phosphorylation of secreted osteopontin (100).

8.2.4. Protein engineering

The engineering of proteins has application because of the wide range of functions that can be induced, potentially going beyond the limits of what can be achieved by endogenous molecules. The promise of such approaches has to be balanced by their challenges, which include the potential antigenicity of novel protein sequences and limited control over possible unwanted effects of structures that have no biological correlate. RGD cell adhesion sequences were engineered into the three-dimensional scaffolding of streptavidin. In cell binding assays, rat aortic endothelial cells and human melanoma cells adhered to surfaces coated with either of the two RGD streptavidin mutants. This adherence was inhibited with soluble RGD peptide or with antibodies directed to integrin $\alpha_V\beta_3$, whereas wild-type streptavidin displayed no significant cell binding activity. These results demonstrated that peptide recognition sequences can be engineered into accessible surface regions of streptavidin without disrupting biotin binding properties (101).

8.2.5. Small molecules

To date, drug research has focused on finding agents to cure primary cancers. However, significant mortality of cancer deaths worldwide result when such drugs fail and the cancer metastasizes and the cancerous cells spread through the body via the blood and lymph. Currently no good broad-spectrum anti-metastatic drugs exist that have proven effective. The introduction of a powerful new anti-metastatic drug would constitute a major medical breakthrough. The clinical introduction of a dual-action, broad-spectrum, anti-tumor drug that could simultaneously inhibit cancer cell growth and function as a potent anti-metastatic drug would be of even greater significance. (-)-Agelastatin A is a naturally occurring *Oroidin* alkaloid and may be suitably obtained from extracts of the *Axinellid* sponge *Agelas dendromorpha*. (-)-Agelastatin A has been identified as a potential anticancer agent and shown to inhibit potently the growth of murine and human cancer cell lines at low drug concentrations. The mechanisms by which this agent acts have yet to be delineated, and there was been no prior suggestion that this agent might also have anti-metastatic effects. (-)-agelastatin A was found to act as a powerful repressor of OPN and β-catenin protein expression (102).

8.3. The CD44 receptor as a target in cancer therapy

The influence that the CD44 receptor has on metastasis is based on three familiar modes of action, specifically, over-expression, ligation with OPN and initiation of various signal transduction pathways, all of which were discussed in previoes sections. Oncogenes, specifically the *ras*-oncogene, up-regulate the expression of cell surface CD44 protein (103). This leads to the identification of the adenoviral gene product, EIA, which prevents the *ras*-induced up-regulation of CD44 receptor. *Ras* inhibitors are currently under evaluation as future anticancer therapy drug. The use of antibodies and peptide antagonists to inhibit binding of CD44 with OPN has also

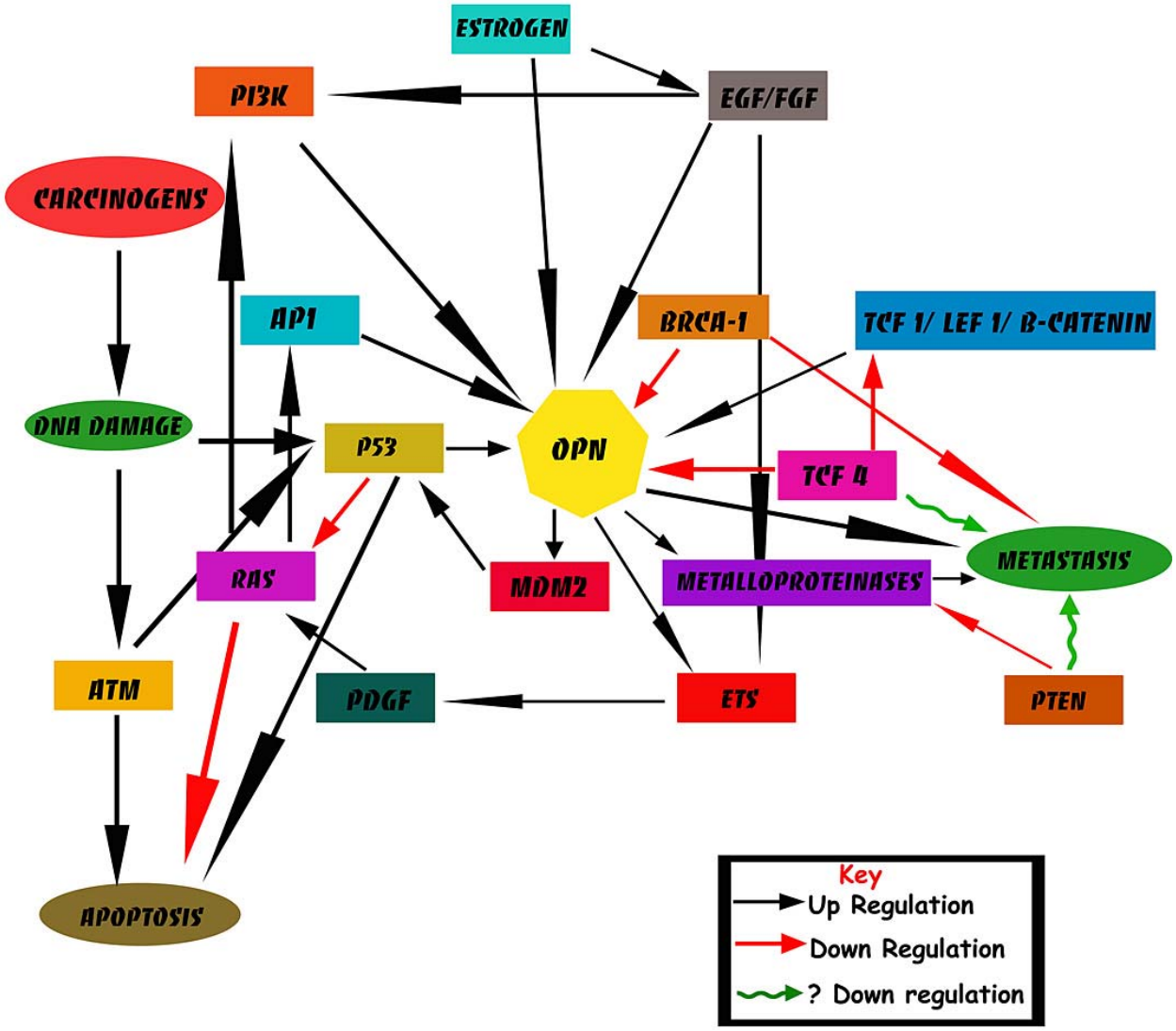

Figure 1. The network of interactions of osteopontin.

been explored and some success in suppressing OPN-induced CD44-mediated tumor progression has been reported. Suppression of the matrix metallo proteinases and uPA are also considered as potential therapeutic targets (104).

8.4. The integrin A_VB_3 receptor as a target in cancer therapy

$\alpha_v\beta_3$ integrin plays an integral role in the complex network of processes that are involved in tumor progression and metastasis, and as such is a prime target for cancer therapy, by halting signal transduction pathway activation which occurs when the OPN binds to integrin. The glucocorticosteroid dexamethasone has been used in an attempt to down-regulate the expression of the $\alpha_v\beta_3$ integrin and there has been some success with its use over a long-term period (96). A reduction in the expression of the integrin resulted in a decrease in tumor cell dissemination. The strategies for inhibiting receptor ligation and signal transduction pathways are based on the use of antibodies and synthetic peptides. Of particular interest is the monoclonal antibody LM609 that acts as an $\alpha_v\beta_3$ integrin antagonist and reduces cell adhesion (105). This antibody also has the ability to cause tumor regression in a single dose by inducing apoptosis of the proliferative angiogenic blood vessel cells (106). Jain *et al* (2007) have provided a useful summary of some of the inhibitors that might be used to regulate the OPN-integrin induced signal transduction pathways in an attempt to prevent tumor progression and metastasis (104).

In cell adhesion, the binding of the RGD domain to integrin is essential. This adhesion property can be inhibited using blocking antibody binding in the vicinity of the RGD site in OPN using specific anti-integrin antibodies. It can also be inhibited using site-directed mutagenesis of the RGD sequence to RGE (107). Substantial research is ongoing into the specific

interaction of OPN with integrins as a therapeutic target, showing it plays an integral role in the tumorigenesis and metastasis processes associated with malignant cancers.

9. PERSPECTIVE

This report began with a brief introduction to osteopontin, and its expression and role in normal physiological processes. These normal processes were then related to similar biological processes that occur in cancer progression. The significance of OPN action, directly and indirectly at each stage should therefore be clear and establishes osteopontin and the downstream signaling pathways it activates as serious potential therapeutic target. Much research is currently being carried out into inhibiting OPN action in cancer in the hope that it will prevent tumor progression and metastasis in the future.

OPN as a gene with a role in cancer was then reviewed and controlling its expression was identified as a potential means of inhibiting its post-transcriptional roles. Hypothemycin and ribozyme, currently undergoing clinical investigations show progress to date. We then focused on OPN as a protein and in the course of the discussion the RGD domain and the thrombin cleavage site were highlighted as being particularly significant. The interaction of OPN with the cell surface receptors integrin $\alpha_v\beta_3$ and CD44 was described. It is clear that the various OPN-receptor interactions are extremely important in tumorigenesis and metastasis. Numerous therapeutic targets were identified here. These included the receptors themselves, including down regulating their expression, inhibiting OPN binding to cognate receptors, and inhibiting the signals produced after OPN interaction with the same receptors.

The review also looked at OPN in specific cancers. Elevated OPN levels in cancer patients are seen to accompany the observation of more aggressive tumors. Recent innovative research has brought about the very real possibility of using OPN as a diagnostic/prognostic marker in the future where patients with high levels of OPN will receive different treatment regimes to those with lower levels. By pursuing this line of attack against cancer it is hoped that more appropriate treatments with less side effects will be created.

The challenge for cancer researchers in the future is to move the basis of work from proof-of-principle (that osteopontin contributes to the metastatic phenotype) to a translational approach of target and drug discovery. Further analysis of the regulation of osteopontin expression, and dissection of the signal transduction pathway downstream, is required. New data will need to be integrated into what we currently know about cancer cell signaling and gene expression programs. Novel processes will thus be revealed, and potential targets for cancer treatment can be identified.

10. ACKNOWLEDGEMENTS

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