

Role of non-collagenous bone protein in metastasis-Pathological basis

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Abstract: Despite modern intervention, the 5-year survival rate of various carcinomas has improved only marginally over the past few decades. Treatment failure for body cancers can be attributed to multiple factors which are difficult to predict. The prognosis largely remains uncertain. Recent studies have focused on the use of biomarkers and gene array technology for determining prognosis in these patients. Non-collagenous bone proteins play an important role in immune mechanisms and helps in regulatory pathways of the body. These induce endothelial cell migration and in macrophages, regulate migration towards some chemokines. Osteopontin plays an important role in the process of angiogenesis. Several studies suggest that osteopontin increases invasiveness by inducing proteinases, and acts in association with other growth factors to induce malignant properties. Many studies indicate that osteopontin expression renders cells more tumorigenic & increases tendency of cells towards metastasis. Osteopontin protein was shown to be elevated in histological sections of several types of human pre-cancer

tissues compared to normal tissue. This article discusses role of osteopontin and other bone proteins in tumorogenesis.

Key-words: *spp* gene, Dentin phosphoglycoprotein, Osteopontin, Biomarkers, Glycoproteins

1. INTRODUCTION

Various biological markers are used for the assessment of cancer risk. Identification of these molecular markers has shown promise as these are used for the diagnosis and future prognosis of malignancies. The recognition of some of the markers are thought to be important with reference to dysplasia's since the reactivity and recognition of these molecules were positively related to different grades of epithelial dysplasia.

The best-characterized markers for determining future cancer development in oral pre-malignant lesions are: Genomic markers that include DNA content (ploidy), chromosome aberrations (allelic loss or gain) and changes in the expression of oncogenes and tumor suppressor genes, Proliferation markers & Differentiation markers including keratins and carbohydrate antigens. Analysis of mutations in tumors, particularly the mutations of p53 gene, is useful tool in the epidemiology of human cancer for several reasons. P53 mutations are common in most types of cancer and p53 proteins typically have a much longer half-life than the wild-type protein, the diagnosis of cancers that harbor mutant p53 is feasible by immunohistochemical detection of its accumulation within the cell.

Iwasa[1] M *et al.*, (2001) the proportion of lesions positive for PCNA, p53 and values of AgNORs parameters steadily increased from hyperplasia to mild, moderate and severe dysplasia, and SCC. Piattelli[2] A *et al.*, (2002) investigated the expression and relationship of p53, bcl-2, MIB-1 and the apoptotic index (AI) in normal oral epithelium, leukoplakia and oral squamous cell carcinoma. They found a strong correlation between p53 over expression and cell proliferation (MIB-1) and the AI. An inverse relationship was found between bcl-2 expression and MIB-1 and AI. A significant inverse relationship was also found between p53 and bcl-2. Kovesi[3] (2002) observed immunohistochemical reactions of Ki-67 and p53 in 15 leukoplakias and 3 OSCC. They examined that the severity of dysplasia, mitotic and apoptotic index and expression as well as distribution of Ki-67 and p53 were related to the clinical appearance of leukoplakia. They found that mitotic index, apoptotic index and Ki-67 expression were increased significantly in parallel with the severity of dysplasia and also with the clinical stage (homogenous, nodular and erythroleukoplakia). Farrar M (2004) applied a panel of monoclonal antibodies (AE1/AE3, cytokeratin [CK] 14, Ki-67 and p53) to 10 cases of human oral tissue in each of six categories to establish staining patterns indicative of its likely progression to malignancy. The six tissue categories were the normal tissue, abnormal benign lesions, mild, moderate and severe dysplasias and SCC. The results showed that AE1/AE3 and CK 14 expression was reduced particularly in poorly differentiated SCC presuming it to be a late event in oral carcinogenesis. Expression of Ki-67 and p53 proved to be a weak but statistically significant predictor of malignant progression in oral tissue.

2. BONE PROTEINS

Osteopontin (OPN), Bone sialoprotein (BSP), Dentin Matrix Protein (DMP), Dentin Sialoprotein (DSP), Matrix Extracellular Phosphoglycoprotein (MEPG) are important non-collagenous proteins which play key roles in the mineralisation of tissues. The genes coding for these proteins family are all located on human chromosome 4q21-23. These proteins have RGD sequences which are shown to bind and activate some matrix metalloproteinases. Since MMPs are critical for development, wound healing and progression of cancer, therefore, the family is considered to have an important role about tumor invasion and metastasis. **Bone Sialoprotein, Osteopontin, Dentin Matrix Protein 1** specifically bind to pro MMP-2, pro-MMP-3, pro-MMP-9 respectively, thereby activating latent proteolytic activity. SIBLING production by tumors could facilitate angiogenesis because both bone sialoprotein (BSP) and osteopontin (OPN) have been shown to possess angiogenesis activity in vitro. As part of SIBLING family, this group of proteins is considered to play a definite role in tumor progression [4], [5].

2.1 Co-distribution among bone proteins: Bone Sialoprotein (BSP) and Osteopontin are found in mineralisation foci near the mineralisation front, accumulate within the spaces between calcified collagen fibrils, and are associated with cement lines. Both of these are multi-functional molecules associated with cementum formation during development and repair tissues. Present data suggest that osteopontin is involved in

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regulating mineral growth, whereas bone sialoprotein promotes mineral formation onto root surface, the balance between the activities of these two molecules may contribute to establishing and maintaining an unmineralised periodontal ligament between cementum and alveolar bone. [8]

2.2 Glycoproteins as related to non-collagenous bone proteins: Among multiple receptors for these proteins, CD44 is the most characterized receptor that appears to mediate chemotaxis and attachment. It plays various biological roles for host defense, bone formation, osteoclast activation and wound healing. Its cytokine activities include the stimulation of macrophage and T-cell migration, protection against herpes viruses and bacterial infections through the activation of the Th1 response, and induction of Th1-cell-mediated autoimmunity. CD44 is a cell surface glycoprotein that serves as an adhesion molecule in cell-substrate or cell-cell interactions[30]. It is strongly upregulated in acute and chronic inflammation. Its ligands include extracellular molecules, like hyaluronic acid and chondroitin sulfate, which can inhibit the cell-cell interactions that lead to macrophage fusion. The widely expressed standard form of this transmembrane protein is CD44s, but a number of splice variants are known that differ in the combinations of additional exons represented in their extracellular region. These CD44 isoforms serve diverse functions. CD44v6 expression on multiple myeloma cells is increased in the bone marrow microenvironment, where it aids in adhesion of the cells. The CD44v7 variant isoform appears to mediate inflammatory bowel disease. CD44v6 and CD44v7 are the principal isoforms able to bind osteopontin. Osteopontin binds the CD44 ezrin/radixin/moesun (ERM) complex in fibroblasts [29]. The osteopontin in the

cytoplasm of tumor cells binds to CD44 on the plasma membrane, transducing the intercellular signal for cell motility. Teramoto *et al.* in their study showed that the OPN-CD44 Rac autocrine pathway was enhanced by H-Ras in NIH3T3 cells. According to them, the ERM binds to actin filaments and induces cell motility. They concluded that osteopontin has the potential to invade tissues through both extra- and intercellular pathways.

3. OSTEOPONTIN AS IMPORTANT NON-COLLAGENOUS BONE PROTEIN

Osteopontin was found in bone matrix by **Herring** in 1983 and plays an important role in anchoring osteoclasts to the mineral matrix of bone. It is a major sialoprotein of the extracellular matrix (ECM) that binds calcium and functions in early-stage mineralization in bone and dentin. Osteopontin has strong affinity for hydroxyapatite, which leads to its accumulation in bone and other sites of mineralization. The word “osteopontin” is derived from two words, *osteo* and *pontin*. The word *osteo* indicates that protein is mainly expressed in bone and the word *pontin* is derived from pons, the latin word for bridge. It is also known by names **sialoprotein I**, **44K BPP** (bone phosphoprotein) and **Early T lymphocyte activation factor**. The osteopontin gene is *secreted phosphoprotein 1 (spp1)* [6]. Located on long arm of chromosome 4, it has 7 exons, 6 of which contain coding sequences & spans 5 kilobases in length. The exons 3, 4, 5, 6, 7 code for 17, 13, 27, 14, 108, 134 amino-acids respectively [7]. The gene expression of osteopontin is upregulated in specific *phases* of osteoblastic lineage differentiation. This gene is expressed during embryogenesis, wound healing, and tumorigenesis.

3.1 Secretion of osteopontin: Osteopontin is secreted by osteoblasts and osteocytes. It is secreted also by fibroblasts, odontoblasts, some bone marrow cells, hypertrophic chondrocytes, dendritic cells, neutrophils, macrophages, monocytes, mast cells, endothelial cells, smooth muscle, skeletal muscle myoblasts, extrasosseous (non-bone) cells in the inner ear, brain, kidney, and placenta[9-14]. It is also abundantly expressed by lesion-associated macrophages in a variety of injury models of heart, brain, and skin. *Runx* and *osx* transcription factors are required for expression of osteopontin [15]. These genes also bind osteoblast specific genes and upregulate transcription [16]. Various phosphate groups have been found to induce osteopontin gene expression [17]. The breast cancer patients are known to express splice varieties of osteopontin [18], [19]. In certain conditions, like hypocalcemia and hypophosphatemia, calcitriol is produced which affects production of osteopontin. This is due to presence of a highly specific vitamin D response (VDRE) in osteopontin gene promoter [20] [21]. The extracellular inorganic phosphate acts as a modulator of osteopontin expression [22]. The stimulation of osteopontin is also due to exposure of cells to pro-inflammatory cytokines[23] and mediators of acute inflammation[24], [25]. Hyperglycemia and hypoxia are known to increase osteopontin expression [26].

3.2 Structure of osteopontin: Osteopontin is highly negative charged extracellular matrix protein, composed of 314 amino-acids. It lacks secondary structure [27]. Starting from N residues, osteopontin contains a motif with 7–10 consecutive aspartic acid (depending on the species), has got alpha helix and heparin binding site, a cell-binding sequence **Arginyl-glycyl-aspartic acid (RGD)**, a thrombin cleavage site, which is six

amino acid residues downstream of the RGD motif, a cryptic epitope (SLAYGLR in mouse and SVVYGLR in human) postulated to be involved in angiogenesis (Hamada *et al.* 2004), a calcium-binding region, and a hyaluronic acid receptor (CD44)-binding domain (Wai and Kuo 2004; Rangaswami *et al* 2006). Depending on the tissue site or factors that regulate its expression, OPN can undergo post-translational modification. Binding of it to the cell surface integrin promotes **Glycine-Arginine-Aspartate-Serine** dependent cell adhesion, stimulates cell migration, activates intracellular signaling pathways involving Phospholipase C, alters intracellular calcium (Ca^{2+}) levels, and activates tyrosine kinases. In addition, osteopontin can be highly phosphorylated on serine and threonine residues. The combination of electronegative glutamic and aspartic acid residues, serine/threonine kinase substrate sites, and the putative calcium-binding motifs endow osteopontin with the ability to bind significant amounts of Ca^{2+} (50 mol calcium to 1 mol osteopontin). These properties contribute to its ability to bind and regulate apatite crystal growth, and the formation of predominant calcium-phosphate mineral phase found in bones and teeth as well as at sites of ectopic calcification. Full length osteopontin (OPN-FL) can be modified by thrombin cleavage, which exposes a cryptic sequence, SVVYGLR on the cleaved form of the protein known as OPN-R. This thrombin-cleaved OPN (OPN-R) exposes an epitope for integrin receptors of $\alpha 4\beta 1$, $\alpha 9\beta 1$, and $\alpha 9\beta 4$ [69]. Integrin receptors are present on a number of immune cells such as mast cells, neutrophils, and T cells. Upon binding these receptors, cells use several signal transduction pathways to elicit immune responses. OPN-R can be further cleaved by

Carboxypeptidase B (CPB) by removal of C-terminal arginine and become OPN-L. The function of OPN-L is not known.

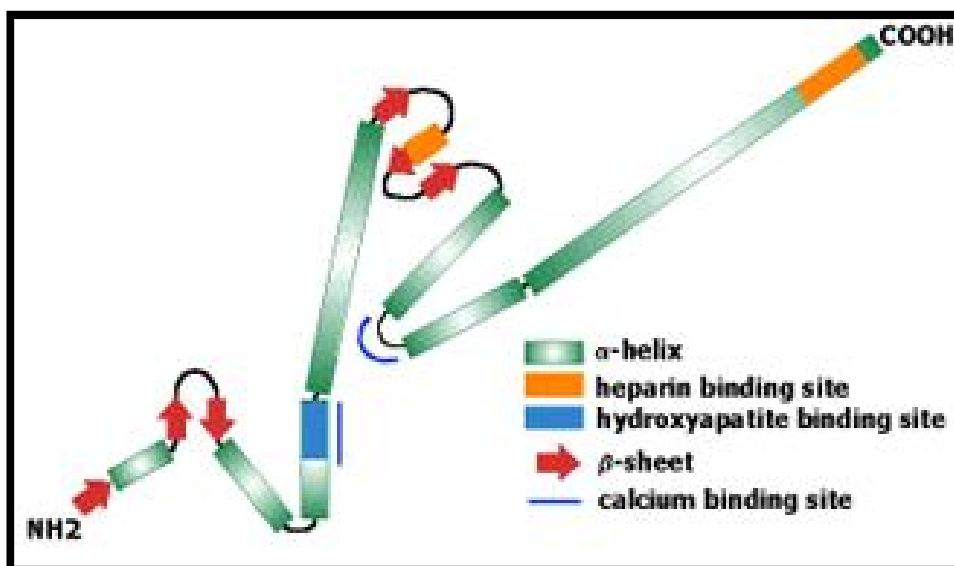


Figure 1: Structure of osteopontin

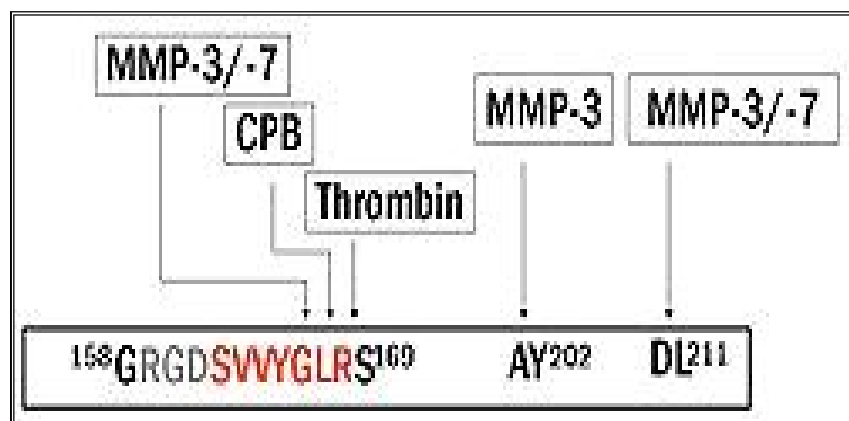


Fig:2 Proteolytic cleavage sites for full length osteopontin (OPN-FL)

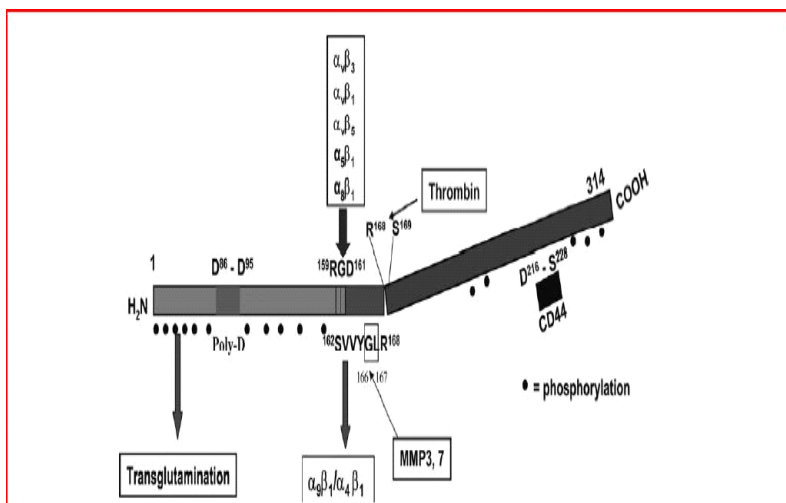


Fig:3 Phosphorylation,integrin binding sites and transglutamination sites

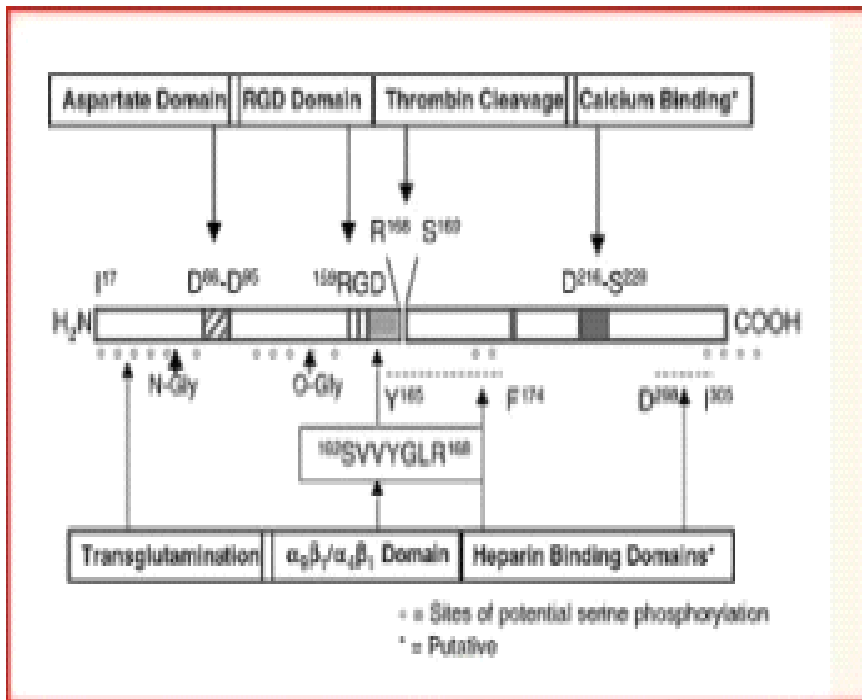


Fig:4 Various domain and cleavage sites of osteopontin

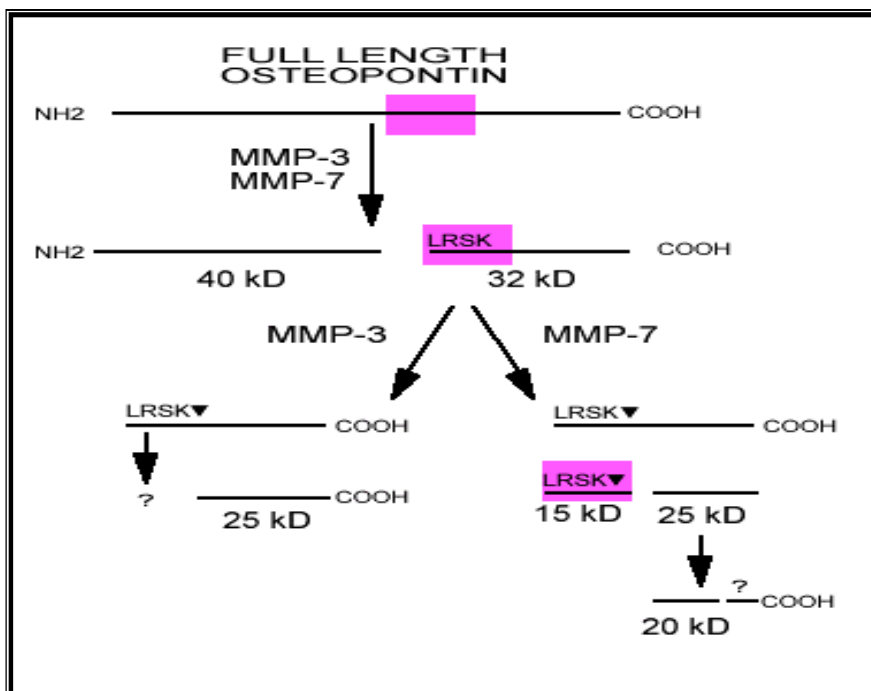


Figure:5 Cleavage sites of osteopontin

3.3 Osteopontin in serum

Normal level of serum osteopontin is about 14-64 ng/ml and if serum level exceed 64 ng/ml, there are increased chances towards metastasis. Increased levels of osteopontin have been found in patients with tumor cell diffusion. So osteopontin is also treated as a serum marker of tumor diffusion. Osteopontin plasma levels have been found to be elevated in head and neck squamous cell carcinoma patients with hypoxic tumors. Patients with higher osteopontin levels appeared to have highly aggressive tumors compared with those with low levels. A significant correlation between plasma OPN level and risk of tumor relapse has been observed. It is believed that there may be a possible link between osteopontin and tumor aggressiveness through tumor hypoxia.

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Osteopontin plasma levels may serve as a potential prognostic factor for tumor relapse and survival in squamous cell carcinoma patients. The over-expression of Osteopontin leads to increase in activation of growth receptor pathways and increased proteolytic enzyme activities. Osteopontin is highly expressed in tumor associated macrophages, the presence of which has been associated with poor outcomes in solid tumors. Fedarko *et al* (2001) measured OPN serum levels in patients with breast, colon, lung or prostate cancer compared with normal serum, and found elevated OPN levels in all tumour types except colon cancer. Hotte *et al* (2002) conducted a prospective study and examined OPN plasma levels in a series of 100 men with hormone refractory prostate cancer. Osteopontin levels were found to correlate negatively and independently with patient survival. In these patients there also was a statistically significant correlation between OPN levels and the presence of metastases to bone.

3.4 Metastatic gene osteopontin

The metastasis gene osteopontin is subject to alternative splicing, which yields 3 messages, osteopontin- a, osteopontin-b and osteopontin-c. Osteopontin-c is selectively expressed in invasive, but not in noninvasive, breast tumor cell lines, and it effectively supports anchorage independence. Osteopontin-b is not a tumor marker. Its contributions to cancer remain to be defined. Osteopontin-c is a better breast cancer marker than osteopontin-a, because it is absent from normal breast tissue.

4. MOLECULAR MECHANISMS IN TUMOROGENESIS

Osteopontin mRNA and protein were shown to be elevated in histological sections of several types of human cancer relative to normal tissue [31-75]. Osteopontin protein and mRNA has been identified in a series of independent biological models. In a series of 25 lung tumor specimens, osteopontin protein and osteopontin mRNA were elevated in tumor tissue, relative to normal lung tissue, and osteopontin immunopositivity was statistically found to be significantly associated with patient survival (Chambers *et al.*, 1996). A case report of bilateral mammary carcinomas showed that osteopontin tumor cell immunopositivity, as well as p53 immunopositivity, were associated with the tumor that recurred locally and progressed to form metastases in the liver and bone (Tuck *et al.*, 1997). In other study done by Tuck *et al.*, (1998), osteopontin mRNA and protein were detected in both tumor cells and infiltrating inflammatory cells, host immune cells were positive for osteopontin protein in 70% of tumors. Sodek *et al.*, in the year 2000, studied intracellular role of osteopontin and found that osteopontin binds CD44 on the inside face of the cell membrane. Denhardt *et al.*, (2001) found that osteopontin regulates cytokine production by macrophages and concluded that osteopontin expression correlated with prognosis. Osteopontin has been detected in a growing number of human tumor types by various methods like, immunohistochemistry on tumor tissue sections, quantification of osteopontin RNA from tumor tissue or in expression array studies from tumor tissues (Furgeret *et al.*, 2001; Tucket *al*, 2001). In a study done by Rittling *et al.*, (2003) it was found that osteopontin regulates cytokine production and cell trafficking in immune systems. Osteopontin protein has a cryptic $\alpha_9\beta_1$ site, that is functional only after protease cleavage, believed to play an important role in tumor

progression. These non-collegenous bone receptors mediate cell adhesion [86], and play an additional role in regulating migration, by influencing intrinsic behaviour of cells. Also this protein regulates cytokine production by macrophages, and in several diverse systems, it has been shown to act as a survival factor.

Tuck *et al* (1995) studied the effect of OPN on cellular invasiveness and basal OPN expression in members of a progression series of human mammary epithelial cell lines (21PT: immortalized, non-tumorigenic; 21NT: weakly tumorigenic; 21MT-1: tumorigenic, weakly metastatic; MDA-MB-435 cells: tumorigenic, highly metastatic). They used RNA isolation and Northern blot analysis for their study. The two lines which expressed lowest basal levels of OPN (21PT, 21NT) were then examined for up-regulation of invasive behavior in response to exogenous or transfected (endogenous) OPN. Both 21PT and 21NT showed increased invasiveness through Matrigel when human recombinant (hr) OPN was added to the lower chamber of transwells. Both also showed a cell migration response to hrOPN. Populations of 21PT and 21NT cells stably transfected with an OPN-expression vector showed higher levels of cell invasiveness than control vector transfectants. Examination of transfectants for mRNA of a number of secreted proteases showed that only urokinase-type plasminogen activator (uPA) expression was closely associated with OPN expression and cellular invasiveness. They found that the potential mechanism of increased invasiveness of breast epithelial cells in response to OPN is due to increased cell migration and induction of urokinase plasminogen (uPA) expression. Coppola *et al* (2004) used immunohistochemical techniques to detect OPN in tissue sections of 350 human tumors and 113 normal

tissues, from a variety of body sites, using stage oriented human cancer tissue arrays. They found high cytoplasmic OPN staining was observed in 100% of gastric carcinomas, 85% of colorectal carcinomas, 82% of transitional cell carcinomas of the renal pelvis, 81% of pancreatic carcinomas, 72% of renal cell carcinomas, 71% of lung and endometrial carcinomas, 70% of esophageal carcinomas, 58% of squamous cell carcinomas of the head and neck, and 59% of ovarian carcinomas. When considering all sites, OPN expression significantly correlated with tumor stage, OPN score and stage were also significantly correlated for specific cancer sites including bladder, colon, kidney, larynx, mouth, and salivary gland. They concluded that there is strong correlation between pathological stage and OPN across multiple tumor types, which suggests a role for OPN in tumor progression.

5. OSTEOPONTIN EXPRESSION IN VARIOUS MALIGNANCIES

5.1 CNS: Increased levels of osteopontin expression correlate with increased tumor grade. Selkirk *et al* (2008) used osteopontin CDNA to infect rat sarcoma cells and human derived glioblastoma multiformae cells. They found little migration in cells expressing osteopontin compared to cells used as control. They concluded that high level OPN expression limits the malignant character of glioma cells and that the downstream mechanisms involved represent pathways that may have therapeutic value in the treatment of human CNS malignancies.

5.2 Dermatologic lesions: The sunlight-exposed skin was shown to have higher expression of osteopontin than foreskins normally not exposed to ultra-violet light. Several studies indicate that UVB could indirectly stimulated osteopontin expression.

The human Osteopontin promoter has been shown to consist of both a functional RAS-activated enhancer (Denhardt *et al.* 2003) .Both the initiator and tumor promotion effect of ultra-violet light can induce the activation of RAS and AP-1, which can result in triggering OPN expression and secretion into the microenvironment of the initiated and normal keratinocytes. Osteopontin on binding to appropriated cell surface receptors enhances the survival of UVB-induced initiated keratinocytes and consequently facilitate the development of Actinic keratosis and Squamous cell carcinomas. Ultra violet light B may also indirectly stimulate osteopontin expression through mutated p53, which is commonly found in 90% of cutaneous SCC and 50% of AK (Brash *et al.* 1996). Elevated expression of osteopontin has been shown to be associated with p53 mutation (Pan *et al.* 2003; Graessmann *et al.* 2006) and p53-null mesenchymal stem cells (Tataria *et al.* 2006). However, osteopontin has been reported to be transcriptionally regulated by TP53 in embryonic fibroblasts (Morimoto *et al.* 2002). Osteopontin also gets activated by UVB-induced production of vitamin D3, which can be converted to the active form 1 α , 25-dihydroxyvitamin D3 (calcitriol) in the skin. This in turn induces the synthesis and secretion of OPN in murine epidermal-like cells (Chang at al., 1991).

5.3 Melanomas: Osteopontin is one of the most abundantly expressed genes in metastatic melanoma nodules. Immunohistochemistry staining on tissue microarrays and individual skin biopsies representing different stages of melanoma progression revealed that osteopontin expression is first acquired at the step of melanoma tissue invasion. The blocking of OPN expression by RNA interference reduced melanoma cell numbers in

vitro. Thus osteopontin may be acquired early in melanoma development and progression, and may enhance tumor cell growth in invasive melanoma.

Kadkol *et al* studied the expression of osteopontin in uveal melanomas. They found that highly invasive primary and metastatic uveal melanoma cells expressed 6- and 250-fold excess osteopontin mRNA, respectively, compared with poorly invasive primary uveal melanoma cells. Tissue sections of primary uveal melanomas lacking looping vasculogenic mimicry patterns either did not stain for osteopontin or exhibited weak diffuse staining. In primary melanomas containing looping vasculogenic mimicry patterns, strong osteopontin staining was detected in the tumor periphery where patterns were located. Diffuse strong expression of osteopontin was detected in eight samples of uveal melanomas metastatic to the liver. Serum osteopontin levels were significantly higher in patients with metastatic uveal melanoma than in patients who had been disease free for at least 10 years after treatment or in age-matched control subjects. Serum osteopontin levels were significantly higher after metastasis than before the detection of metastasis in eight patients. When a cutoff of 10 ng/ml was used, the sensitivity and specificity of serum osteopontin in detecting metastatic melanoma was 87.5%, and the area under the receiver operator characteristic curve was 96%.

5.4 Renal cell carcinomas: Osteopontin expression has been associated with liver cirrhosis. Koviljka *et al.*, analysed the expression of osteopontin protein immunohistochemically in 171 renal cell carcinomas and compared to usual clinicopathological parameters such as tumor size, nuclear grade, pathological stage, Ki-67 proliferation index, and cancer-specific survival. They found that in normal renal

parenchyma, the expression of osteopontin was seen in distal tubular epithelial cells, calcifications, and some stromal cells. The upregulation of osteopontin was observed in 61 renal cell carcinomas (35.7%) in the form of cytoplasmic granular staining of various intensities. Moreover, patients with OPN-positive tumors had significantly worse prognosis in comparison to patients with tumors lacking osteopontin protein. These results suggest that overexpression of osteopontin is involved in the progression of renal cell carcinomas. Brown *et al.*, analyzed distribution of OPN mRNA and protein in 14 RCCs, and found strong expression of OPN mRNA in 13 cases, and strong and diffuse cytoplasmic staining for OPN protein in 7 cases. In their study, all tumors were moderately differentiated CRCC, except for one well-differentiated papillary carcinoma, which was also positive. Matusan *et al.*, (2006) in their study found that the level of OPN expression strongly correlated with tumor variables like; histological grade, pathological stage, tumor size, and Ki-67 proliferation index. While all of grade 1 tumors were negative for OPN protein, the positivity increased with transformation to higher nuclear grade. They concluded that the upregulation of osteopontin protein in a large group of renal cell carcinoma patients was associated with poor prognosis.

5.5 Bone tumors: Many studies indicate expression of OPN in bone tumors. Jan lisch Fischer (2001) found increased expression of osteopontin in mice which were experimentally induced with osteosarcomas. They also found increased expression of osteopontin by organs which got metastasized subsequently.

Liu si jin *et al* (2004) explored the effect of osteopontin on the proliferation, transmigration and expression of MMP-2 and MMP-9 in osteosarcoma cells in vitro. The

expression of MMPs was evaluated by detecting the volume of degradation of gelatin on SDS-PAGE gel. The secretion of MMPs particularly MMP2 AND MMP-9 was found to be more than normal. They found that osteopontin stimulated cyclin A expression in these cells to accelerate cell division cycle. Osteopontin was found to be chemotactic for osteosarcoma cells and thus facilitate transmembrane migration of osteosarcoma cells .They also found that osteopontin promoted proliferation of osteosarcoma cells in a dose dependent manner.

Youqiao *et al.*, (2007) conducted studies to detect the expression of osteopontin in osteoblastomas and osteosarcomas. Osteoblastoma osteoblasts as well as osteoclast-like giant cells and osteosarcoma mononuclear cells showed variable staining. In one study, osteosarcoma, COX-2 expression and osteopontin correlated with each other. Although osteosarcoma patients with high COX-2 expression showed a trend towards shorter overall survival, osteopontin expression had no influence on patients overall or on disease-free survival. The relation between osteosarcoma cells correlated with expression of vascular endothelial growth factor (VEGF) in presence of osteopontin, but no independent relation between osteosarcoma and osteopontin was elucidated.

5.6 Malignant mesotheliomas: Zhao[125] *et al* showed that osteopontin could be used to evaluate the existence of liver cirrhosis; this unique feature makes OPN a promising candidate for prediction biomarker in the long-time surveillance of patients with HBV infection to evaluate the risk of cirrhosis and cancer. Pass *et al.*, (2005) at Wayne state university found that malignant mesothelioma patient had osteopontin levels that were approximately six times higher than normal levels. These studies indicate that

osteopontin can play an important role in the diagnosis of malignant mesotheliomas in future.

5.7 Ovarian carcinomas: Increased OPN expression has been found to be related to ovarian carcinomas. Kim *et al.*, (2007) conducted experimental and cross-sectional on tissue samples of patients who ovarian cancer and healthy human subjects. Also plasma samples from 107 women selected from an epidemiologic study of ovarian cancer were used as healthy controls. Relative messenger RNA expression in cancer cells and fresh ovarian tissue, measured by real-time polymerase chain reaction was measured. Osteopontin production was localized and scored in ovarian healthy and tumor tissue with immunohistochemical studies. The amount of osteopontin in patient *vs* control plasma was measured using an enzyme-linked immunoassay. Immunolocalization of osteopontin showed that tissue samples from 61 patients with invasive ovarian cancer and 29 patients with borderline ovarian tumors expressed higher levels of osteopontin than tissue samples from 6 patients with benign tumors and samples of healthy ovarian epithelium from 3 patients. Osteopontin levels in plasma were significantly higher in 51 patients with epithelial ovarian cancer (486.5 ng/mL) compared with those of 107 healthy controls (147.1 ng/mL), 46 patients with benign ovarian disease (254.4 ng/mL), and 47 patients with other gynecologic cancers (260.9 ng/mL). They concluded that there is an association between levels of osteopontin, and ovarian cancer.

5.8 Auto-immune disorders: High levels of osteopontin have also been observed in many autoimmune diseases, such as systemic lupus erythematosus, rheumatoid arthritis and multiple sclerosis. Hur *et al.*, in a study of multiple sclerosis patients found that

osteopontin triggered recurrent relapses, promoted worsening paralysis and induced neurological deficits, including optic neuritis. They found increased inflammation followed administration of osteopontin. The absence of osteopontin resulted in more cell death of brain-infiltrating lymphocytes. According to them, osteopontin promoted the survival of activated T cells by inhibiting the transcription factor Foxo3a, by activating the transcription factor NF- κ B through induction of phosphorylation of the kinase IKK and by altering expression of the proapoptotic proteins Bim, Bak and Bax. These mechanisms collectively suppressed the death of myelin-reactive T cells, linking osteopontin to the relapses and insidious progression characterizing multiple sclerosis.

Many studies reveal role of osteopontin in arthritis. **Petrow *et al.***, (2000) studied the expression of OPN messenger RNA (mRNA) and protein in synovia from 10 Rheumatoid arthritis patients by in situ hybridization and immunohistochemistry. Synovial fibroblasts from rheumatoid arthritis patients and articular chondrocytes from patients without joint disease were cultured in the presence of various concentrations of OPN, and levels were measured by enzyme-linked immunosorbent assay. The expression of OPN mRNA and protein was observed in 9 of 10 specimens obtained from patients with rheumatoid arthritis. OPN was expressed in the synovial lining and at the interface of cartilage and invading synovium. Double labeling revealed that the majority of OPN-expressing cells were positive for the fibroblast-specific enzyme prolyl 4-hydroxylase and negative for the macrophage marker CD68. OPN staining was not observed in lymphocytic infiltrates or leukocyte common antigen (CD45) positive cells. Three of 3 cultures of human articular chondrocytes secreted detectable basal amounts of collagenase, with a dose-dependent

increase upon OPN stimulation, while synovial fibroblast cultures produced much lower levels of collagenase, with only 2 of 4 fibroblast cultures responding in a dose-dependent manner. They concluded that osteopontin produced by synovial fibroblasts in the synovial lining layer and at sites of cartilage invasion not only mediates attachment of these cells to cartilage, but also contributes to matrix degradation in rheumatoid arthritis by stimulating the secretion of collagenase 1 in articular chondrocytes.

6. CONCLUSION

Further studies on large number of samples on different degrees of dysplasia, carcinoma in situ, OSMF and different histological grades of OSCCs with clinical and patient outcome data, along with pro angiogenic markers may help in determining the diagnostic and prognostic value of osteopontin.

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