Serum Osteopontin as a Potential Marker for Metastasis and Prognosis in Primary Osteogenic Sarcoma: A Systematic Review

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Abstract

Osteogenic Sarcoma is a painful medical burden, and treating it is still a difficult issue. Therefore, there is an urgent need for advancements in Osteosarcoma diagnosis and treatment.

Comprehensive literature searches were conducted in the PubMed databases for research published between the database’s establishment and November 11, 2022.

Out of the nine studies which were available for analysis, higher level osteopontin in primary osteogenic sarcoma patients indicates a poorer prognosis, higher incidence of metastasis but per say using Osteopontin as a marker for diagnosis of osteosarcoma seems inconclusive.

Data extracted from previously published studies suggest that initially in primary osteogenic sarcoma patients, Osteopontin level are low for increase proliferation of immature osteoblast but late on for survival of tumour (for 1 cm) Osteopontin helps in neovascularization and thereby helps in metastasis. Therefore, Serum Osteopontin level might not be reliable marker for diagnosis of primary osteogenic sarcoma but it is useful marker for predicting metastasis and prognosis.

Whilst an early diagnosis and better treatments have increased the 5-year survival of other tumors, the result for Osteosarcoma has not shown a commensurable progress. Therefore, there is an urgent need for advancements in Osteosarcoma diagnosis and treatment. A ground-breaking development for the diagnosis of Osteosarcoma would be the discovery of a biological marker that can predict metastasis and severity.

Categories: Oncology, Orthopedics
Keywords: osteosarcoma, os, opn, primary osteogenic sarcoma, osteopontin

Introduction And Background

• Osteosarcoma

The most common bone cancer and the second-leading cause of cancer-related deaths in children and adolescents is osteosarcoma (OS)[1].

The incidence rates of Osteosarcoma is 4 and 5 cases per million people per year, respectively, for ages 0-14 and 0-19. In India, where this cancer is associated with higher deaths, the incidence ranges from 4.7% to 11.6%[2]. Males are more likely than females to develop OS than vice versa (5.4 vs. 4.0 cases per million individuals per year, respectively). The incidence of OS has two peaks, the first of which occurs between the ages of 10 and 14, during period of rapid growth, showing a strong correlation between adolescent and OS. Above 65 years, the second peak occurs often secondary to Paget’s disease. The long bones—including the proximal tibia and distal femur—are where majority of OS originate. OS is extremely aggressive and has a 20% metastatic rate, with the lungs being the typical site of metastasis [3]. Figure / below depicts data derived from the Surveillance, Epidemiology, and End Results (SEER) program on the population, previously published by Mirabello et al.
The mainstay treatment is surgery with excision of primary lesion and occasionally distant metastatic tumours with or without adjuvant or neoadjuvant chemotherapy. Surgical procedure includes amputation or salvage. Chemotherapy Regime can be in form of neoadjuvant which includes 4 cycles dexamethasone, Cisplatin, Adriamycin and Ifosfamide with cycle interval of 3 weeks or adjuvant which includes 4 cycles of ifosfamide and cisplatin with cycle interval of 3 weeks. However, early metastasis can lead to treatment failure and death. The prognosis with primary tumor alone is significantly better as compared to patients with metastasis. In patients with metastases at baseline, the 5-year survival rate was 27.4% and 70% for patients with no metastases [5].

• Osteopontin

Osteopontin (OPN) is a chemokine-like calcified extracellular matrix-associated protein first identified in bone [6]. The 314 amino acid residue human OPN is a highly negatively charged protein that appears to have no complexity in its secondary structure [7]. Serine-valine-valine-tyrosine-glycine-leucine-arginine and arginine-glycine-aspartate domains for human OPN integrin binding, calcium binding sites, extracellular matrix receptor III (CD44 antigen-mediated) binding [8].

Osteopontin is a protein which helps in maturation of osteoblast with the help of Run related transcription factor 2 converting the mesenchymal stem cells to mature osteoblast. Due to dysregulation of expression of tumor suppressor genes triggered by genetic or epigenetic events or mutation creates microenvironment for the osteosarcoma by decreasing the Osteopontin level thereby preventing maturation of the osteoblast as shown in Figure 2. This leads to proliferation of immature cells. But as the tumour grows, it needs neovascularization to prevent the hypoxic condition causing the release of Hypoxic inducing factor which increases the level of OPN and promotes neovascularization.
FIGURE 2: Flowchart depicting OPN relation with OS

Abbreviations: S100A4: S100 calcium binding protein A4; CD44: Extracellular matrix receptor III; VEGF: Vascular endothelial growth factor; MMP: Matrix metalloproteinases; GLUT 1/3: Glucose transporter 1/3; OPN: Osteopontin; OS: Osteosarcoma.

Hence the elevated level of OPN and subsequent neovascularization stimulates cascade of events leading to increase in GLUT 1 and GLUT 3 causing more uptake of glucose by tumour cells prolonging their survival and enhancing their proliferation as described in Figure 3.
Osteogenic Sarcoma is a painful medical burden, and treating it is still a difficult issue. Whilst an early diagnosis and better treatments have increased the 5-year survival of other tumours, the clinical outcomes for OS have not demonstrated a commensurable progress. Therefore, there is an urgent need for advancements in OS diagnosis and treatment. A ground-breaking development for the diagnosis of OS would be the discovery of a biological marker that can predict metastasis and severity. The investigation and development of therapeutic approaches for OS patients may be aided by the identification of specific tumour biomarkers.

Therefore, we aimed to systematically review studies investigating role of Osteopontin in Osteosarcoma.

**Review**

**Objectives:**

1. To assess S. Osteopontin level as a marker for severity in Primary Osteogenic Sarcoma
2. To assess S. Osteopontin level as a marker for metastasis in Primary Osteogenic Sarcoma
3. To assess S. Osteopontin level as a marker for Therapeutic outcome in Primary Osteogenic Sarcoma

**Search Strategy:** This study was conducted in accordance with PRISMA guidelines (Preferred Reporting Section for Systematic Review and Meta-analysis). We used the search terms ("osteogenic sarcoma" OR "bone sarcoma" OR "primary osteosarcoma" OR "BONE") AND ("Osteopontin" OR "Sialoprotein 1" OR "secretory phosphoprotein 1") OR "Uropontin" OR "SPP 1" OR "OPN"). Retrieved studies are limited to English only.

**Eligibility Criteria:** All studies showing relation with Osteopontin and osteosarcoma were included and abstracts and animal studies were excluded

**Sources:** On 5th November 2022, we searched PubMed database for Osteopontin and osteosarcoma. We also

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**FIGURE 3: Pathogenesis of osteosarcoma recurrence and metastasis**

Abbreviations: OS: Osteosarcoma; OPN: Osteopontin; S100A4: Calcium binding protein A4; TGFβ1/2: Transforming growth factor β1/2; VEGF: Vascular endothelial growth factor; GLUT1/3: Glucose transporters 1/3; MMP: Matrix metallo proteinases; PI3K: Phosphoinositide-3-kinase; CD44: Extracellular matrix receptor III; AKT: Protein kinase B; p38: Mitogen-activated protein kinase; JNK: c-JUN N-terminal kinase; NF-κB: Nuclear factor-κB.

performed a snowball search to identify additional studies by searching the reference list for publications eligible for full-text review and using PubMed to identify and filter the respective researches citing those references.

**Selection Process:** Two researchers individually reviewed the titles and abstracts of the articles and determined contrariety until a consensus was reached. In the event of disagreement, third-researcher’s consensus is taken on which articles should be screened as full-text. Again, if there is disagreement, consensus on inclusion or exclusion has been reached through discussion.

**Result:** Out of 187 studies from Pubmed database, 100 studies being animal studies were excluded, 78 did not match the objective. 9 studies were considered for full review out of which 2 studies were abstract. The flowchart depicts below the breakdown of databases required to formulate an adequate screening process(Figure 4).

![Flowchart depicting screening of database](image)

**FIGURE 4: Flowchart depicting screening of database**

An overview of the studies selected for this review is summarized in Table 1.
### TABLE 1: A tabulated summary of the selected studies characteristics.

<table>
<thead>
<tr>
<th>Authors List</th>
<th>Study Name</th>
<th>Sample Size/ Sample cell Line</th>
<th>OS patients</th>
<th>Healthy individuals</th>
</tr>
</thead>
<tbody>
<tr>
<td>Han et al [9]</td>
<td>Osteopontin as a biomarker for osteosarcoma therapy and prognosis [9].</td>
<td>40</td>
<td>11</td>
<td>29</td>
</tr>
<tr>
<td>Liang et al [12]</td>
<td>The cancer-related transcription factor Runx2 combined with osteopontin: a novel prognostic biomarker in resected osteosarcoma [12].</td>
<td>105</td>
<td>105</td>
<td>-</td>
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<tr>
<td>Hsieh et al [13]</td>
<td>Osteopontin upregulates the expression of glucose transporters in osteosarcoma cells [13].</td>
<td>Human osteosarcoma cell lines MG63, U-2OS, and 143B [13].</td>
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<tr>
<td>Song et al [14]</td>
<td>Regulation of osteosarcoma cell invasion through osteopontin modification by miR-426 [14].</td>
<td>U2OS is a human OS line [14].</td>
<td>-</td>
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<tr>
<td>Liu et al [15]</td>
<td>Effect of human osteopontin on proliferation, transmigration and expression of MMP-2 and MMP-9 in osteosarcoma cells [15].</td>
<td>OS cell cultured in vitro in 1460 cell culture.</td>
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#### Discussions

- **S. Osteopontin as a marker for prognosis in osteosarcoma**

  Of the studies included in this review, three of them primarily focused on evaluating the potential value of OPN as a biomarker for OS. These studies concluded that OPN may be used as a biomarker for early diagnosis, therapeutic effectiveness and prognosis in OS patients and highlighted the association of OPN with micrometastases in patients with OS. The demographic analysis with detailed description of each is given in Table 2.
The study Osteopontin as a biomarker for osteosarcoma therapy and prognosis [9] given by Xingwen Han et all, showed that osteopontin can be used as biomarker for diagnosis and treatment for various cancer and not just only osteosarcoma but also multiple tumours consisting of ovarian, gastric, lung, breasts and melanoma. It has described the functional and structural characteristics of osteopontin along with concluding the unique role played by Osteopontin in the proliferation and metastasis of malignant cells. The interaction of osteopontin with hypoxia-inducible factor and promotion of E-cadherin to help tumor metastasis is also shown. It also describes that Osteopontin helps in maturation of osteoblast while osteosarcoma is proliferation of immature cell indicating that Osteopontin level should be low initially for proliferation of tumour cell but for cells to metastatize Osteopontin level should be high. It also included a study in which 11 osteosarcoma patients and 29 healthy individuals were taken showed that 91% of osteosarcoma patients Osteopontin levels were high but on other hand in only 35% normal healthy individual it was raised, It was also found that in 6 osteosarcoma patients with relatively higher serum Osteopontin level developed clinical metastasis within 12 months of diagnosis. Even though it combined various studies but did not provide any standard reference value for Osteopontin for which proper case control studies have to be carried out. It also did not clearly tell the relationship of Osteopontin and its role in prognosis of osteosarcoma.

### TABLE 2: Overview of the results of selected studies depicting efficacy of OPN as a biomarker for OS therapy and prognosis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Description</th>
<th>Patient Details</th>
<th>OPN Level</th>
<th>Chemotherapy</th>
<th>Metastasis</th>
<th>OS Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nazarizadeh et al [10]</td>
<td>Evaluation of local and circulating osteopontin in malignant and benign primary bone tumours [10].</td>
<td>72 81 OS: 27</td>
<td>0.22-0.036</td>
<td>0.57-1.02 OS: 0.71-1.02</td>
<td>0.03-0.06</td>
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<td>Metastatic OS</td>
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<td>OPN level (p=0.049)</td>
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<td></td>
<td>Not receiving chemotherapy</td>
<td>Receiving chemotherapy</td>
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<td>OPN level (p=0.237)</td>
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<td>Positive OPN</td>
<td>Negative OPN</td>
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<td>Lung Metastasis</td>
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<td>Yes -34 No-21</td>
<td>Yes -9 No-41</td>
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<td>Tumor size</td>
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<td>&lt;8cm -42 &gt;8cm-13</td>
<td>&lt;8cm-28 &gt;8cm-22</td>
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<td>Histologic response</td>
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<td></td>
<td>Good-27 Poor-19</td>
<td>Good-16 Poor-34</td>
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<td>Surgical Margin</td>
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<td></td>
<td></td>
<td>Wide-43 Marginal-12</td>
<td>Wide-44 Marginal-6</td>
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</tbody>
</table>
Study given by Ali Nazarizadeh evaluates local and circulating osteopontin in benign primary and malignant bone tumors. Serum Osteopontin level was assessed in 153 patients in which 72 had benign primary bone tumor and 81 (out of which 27 were osteosarcoma patients) has malignant primary bone tumor and 29 healthy individuals were taken as control. Results showed that serum Osteopontin level were highest in malignant primary bone tumors followed by benign primary bone tumor followed by controls. It also showed that OPN levels could predict metastasis, grade and prognosis. P value of <0.05 were considered statistically significant. It also showed that OPN mRNA expression in patients with osteosarcoma receiving chemotherapy was 1.5 times lower than the untreated counterparts, Osteopontin level was 1.7 times higher in osteosarcoma patients with metastasis in comparison to osteosarcoma patients with no metastasis. Also, OPN level was 2 times lower in osteosarcoma patient with no recurrence in comparison to patients having recurrence. Only limitation of this study was that it focused on Osteopontin as marker for primary bone tumour was not just specifically for osteosarcoma and the survival analysis was not carried

The study by Liang et al aimed to explore the prognostic significance of the role played by Runt related transcription factor 2/OPN axis in metastasis of OS cells. The study including a total of 105 clinical samples from osteosarcoma patients substantiated the expression of osteopontin and runt-related transcription factor2 through immunohistochemistry. Proceeding analysis of correlations between OPN, Runx2, and clinicopathology was done by Chi-square ($\chi^2$) tests. The prognostic values were determined by univariate and multivariate survival analysis. Further evaluation of the accuracy of ontological outcome prediction was done by Receiver-operating characteristics curves. Results concluded the possibility of a combined expression of Runx2/OPN as a valuable independent predictor of tumor metastasis and survival in osteosarcoma patients. There is a significant positive correlation between Runx2 and OPN expression at protein levels (P = 0.015) [12]. Combined consideration of OPN and Runx2 showed extensions in predictive range as well as an improvement in sensitivity, leading to a probable novel prognostic biomarker. Although the study aims to emphasize the combined role of Runx2 and OPN as a prognostic tool in resected osteosarcoma but sheds limited theoretical explanation about the tumor metastasis and survival. The result lacks to establish the consistency of future trends of outcomes obtained which could be substantiated with a proper patient follow up taken at a time frame of six months.

- S. Osteopontin as a marker for severity in Osteosarcoma

Further studies mentioned in Table 3 demonstrated the relationship of osteopontin with glucose transporters and matrix metalloproteinases both of which are crucial factors in osteosarcoma proliferation and severity along with the determination of therapeutic approaches.
### Authors List

<table>
<thead>
<tr>
<th>Study Name</th>
<th>Sample</th>
<th>Result</th>
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<tbody>
<tr>
<td><strong>Hsieh et al [13]</strong></td>
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<td></td>
<td>OS cell cultured in vitro in 1460 cell culture</td>
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</table>

**TABLE 3: Summary of the results of the selected studies depicting sOPN as a possible marker for severity of osteosarcoma in OS patients.**

| Abbreviations: sOPN: Serum Osteopontin; OS: Osteosarcoma; GLUT 1/3: Glucose transporter 1/3; MG63: Human osteoblastic cell line; U-2OS: Human bone osteosarcoma epithelial cell; 143B: Cell line derivative of HOS cell line; shOPN 1/2: Human osteopontin plasmid 1/2; MMP 2/9: Matrix Metalloproteinases 2/9. |

Matrix Metallo Proteinases have significant roles in growth of tumor cells, migration, invasion, metastasis,
The study conducted by Liu et al explored the effect of human osteopontin (hOPN) on the proliferation, transmigration, and expression of matrix metalloproteinase-2 (MMP-2) and matrix metalloproteinase-9 (MMP-9) in osteosarcoma (OS) cells in vitro. In the study, a prokaryotic-expression vector of hOPN was produced and subcloned into E. coli BL21 (DE3) cells and purified with ProBond trade mark Columns [15]. Furthermore, the proliferation, cell cycle, and the expression of cyclin A in OS cells were investigated by using MTT assay, flow cytometry and Western blot respectively [15]. Transwell cell culture chamber was used to check the transmigration of OS cells. The chemotaxis of hOPN to OS cells was studied by micro-pore-filter-membrane system.

The levels of total protein were examined according to Coomassie Brilliant Blue manuals followed by evaluation of the expression of MMP-2 and MMP-9 by detecting the volume of degradation of gelatin on SDS-PAGE gel. The resultant prokaryotic-expression vector of hOPN and purified hOPN protein were achieved, indicating hOPN promoted OS cells proliferation in a dose-dependent manner along with stimulating cyclin A expression in OS cells to accelerate cell division cycle [15].

hOPN has chemotaxis to OS which increases the trans-membrane migration of OS cells and also enhances the secretion of MMP-2 and MMP-9 in OS cells [15]. The study establishes the correlation between MMP and hOPN in a summarized manner but doesn’t provide an extensive investigation to assess the consistency of outcomes. OS cells in presence of OPN were more in S phase of cell cycle as compared to less number in G0/G1 cycle which helped in multiplication of these cell. But this study was invitro study. No factual explanation about the direct prognostic role of MMP positive expression with respect to OPN in OS is made. The study is exclusively invitro hence in order to strengthen the findings and further test the clinical applications of determining the influence of MMP-2, MMP-9, and hOPN on the metastasis of OS patients, well-designed clinical trials with bigger human sample sizes must be carried out.

The study by Hsieh et al investigates how in osteosarcoma, osteopontin enhances the expression of glucose transporters. The most frequent primary bone malignancy is osteosarcoma. Despite the typical standard surgical therapy, metastasis still occurs in a significant portion of patients. In tumor proliferation and survival, glucose acts as a crucial source of metabolic energy. Typically many types of tumors showcase an overexpression of osteopontin and glucose transporters like GLUT1 and GLUT3 (hypoxia-responsive). Its associated to tumorigenesis and metastasis.

For the study, the human osteosarcoma cell lines MG63, U-2OS, and 143B were purchased from the American Type Culture Collection (Rockville, MD). MG63 cells were cultured with DMEM (Gibco; Grand Island, NY), U-2OS cells were cultured with RPMI 1640, and 143B cells were cultured with MEM. All cell cultures were supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT) and maintained at 37°C in a humidified atmosphere with 5% CO2. An OPN-shRNA (short-hairpin RNA) conjugated to the pLKO.1 vector containing a puromycin-resistant region was provided by the National RNAi Core Facility at the Institute of Molecular Biology/Genomic Research Center in Taipei in Taiwan [13].

Control shRNA (empty vector; ev) was used as a negative control. Cells were transfected with two different OPN-shRNA plasmids for 24 h for transient transfection. CoCl2 was used to mimic the hypoxic condition as an inevitable cellular stress experienced during tumor progression and solid tumor development which upregulated OPN mRNA and protein expression in osteosarcoma cells. It was also demonstrated that GLUTs can be upregulated by both CoCl2 and OPN, hence glucose uptake was evaluated using a fluorescent d-glucose analog, 2-NBDG, in MG63 osteosarcoma cells with confocal microscopy and flow cytometry. The intracellular fluorescence intensity of 2-NBDG was enhanced by treatment with OPN in MG63, indicating that OPN increases nutrient availability to osteosarcoma cells. This effect was mediated by FAK phosphorylation, and the AKT, JNK, and p38 MAPK pathways indicating that these kinases are involved in the regulation of glucose transporters by OPN via α3β3 integrin. Knockdown of OPN expression was performed in the osteosarcoma cell lines MG63 and U-2OS using two different shRNA plasmids which decreased the cell survival by approximately 20% in OPN knockdown cells. Hypoxia-induced expression of GLUTs was also inhibited by OPN knockdown. As a result, it was observed that both glucose transporters and osteopontin were upregulated in hypoxic human osteosarcoma cells.

Subsequently, a glucose transporter inhibitor- Phloretin, also caused cell death by treatment alone.
Comparatively, the phloretin-induced cell death was significantly enhanced in osteopontin knockdown osteosarcoma cells to 80%. Synergistic cytotoxic effects were exhibited in three osteosarcoma cell lines by a combination of a low dose of phloretin and chemotherapeutic drugs such as daunomycin, 5-Fu, etoposide, and methotrexate. Inhibition of glucose transporters markedly potentiated the apoptotic sensitivity of chemotherapeutic drugs in osteosarcoma [13].

These results highlight the significance of combination of low dose of a glucose transporter inhibitor with cytotoxic drugs [13] as a new therapeutic option for treating osteosarcoma patients. However, a cellular based study might lack short in result consistency and sensitivity with control and non-control group patients on a larger sample size. Moreover, the study is based on GLUT-OPN relationship and providing a potential chemotherapeutic treatment for OS and only indirectly provides OPN role in prognosis with respect to other simultaneous factors involved in OS patients.

• S. Osteopontin as a marker for metastasis in Osteosarcoma

One of the studies as mentioned in Table 4 aimed to explore the combined Runx2/OPN expression as a valuable independent predictor of tumor metastasis and survival in osteosarcoma patients.

<table>
<thead>
<tr>
<th>Author Name</th>
<th>Study Name</th>
<th>Sample</th>
<th>Result</th>
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</thead>
</table>

### TABLE 4: Result of study showing Runx/OPN expression in surgically resected Osteosarcoma.

*Abbreviations: RUNX2: Runt-related transcription factor 2; SaOS-2: Sarcoma osteogenic cell line 2; MG63: Human osteoblastic cell line; U2OS: Human bone osteosarcoma epithelial cell; HOS: Human osteosarcoma cell; G292: Human osteosarcoma cell; 143B: Derivative of HOS cell line; OPN: Osteopontin.*

Study given by Francisco Villanueva investigates the cancer related transcription factor RUNX2 modulating expression and secretion of OPN in osteosarcoma cells to promote adhesion to endothelial pulmonary cells and lung metastasis.

The study used Human osteosarcoma cells SaOS-2, MG63, U2OS, HOS, G292, and 143B were obtained from American Type Culture Collection. Adenovirus was used for delivery of vector containing the complementary DNA (cDNA) of Runx2 coupled to a green fluorescent protein (Runx2- IRES-gfp) under control of the CMV5 promoter [11]. Adenovirus infected these osteosarcoma cell line and further experiment was carried out by siRNA. Student T test with p value less than 0.05 were taken. The study shows that that Osteopontin receptors ITGB1, ITGB3, ITGB5, ITGA4, ITGA5, ITGA6, ITGA7, ITGA8, ITGA9, and ITGAV), CD44 and CXCR were expressed on both Osteopontin and pulmonary endothelial cells therefore suggesting that Osteopontin secretion helps osteosarcoma cells to adhere with pulmonary endothelial cells. Osteopontin expression is directly controlled by RUNX2 [11].

The study primarily focused on RUNX2 rather than Osteopontin. It mainly focused on treatments that were targeted towards reducing pulmonary metastasis in osteosarcoma. But gave a indirect clue that increase Osteopontin via RUNX2 mediated pulmonary cell adhesion and caused pulmonary metastasis. It was cell line based study with no proper case and controls being taken. Even though study tells about the lung metastasis and treatment to prevent metastasis but did not comment for metastasis at elsewhere also not deals with the relationship of Osteopontin with severity and prognosis of osteosarcoma.

The study by Song K et al determined the Regulation of osteosarcoma cell invasion through osteopontin.
Disclosures

Additional Information

Conclusions

Primary osteogenic sarcoma being tumor of immature osteoblast but on other hand Osteopontin helps in maturation of osteoblast but their relationship is quite controvertible. Osteopontin expression is essential for maturation of osteoblast via integrin αvβ3-mediated cell signalling, osteopontin is essential for controlling osteoblast development. In comparison to differentiated mature osteoblasts, osteosarcoma cells exhibit much lower levels of OPN expression. Reduced osteopontin levels impede MSCs' ability to differentiate into OBs which is consistent with the finding that tumor is proliferation of cells which are not fully differentiated. Decrease O.P.N. expression impairs the differentiation of mesenchymal stem cells or immature osteoblasts into mature osteoblasts while preserving the characteristics of immature osteoblastic-like cells, which may lead to OS Alteration in serum O.P.N. levels may be linked with differentiation, proliferation and maturation abnormalities in OS cells. Lower levels of O.P.N. expression in OS cells indicate that most OS cells fail to undergo terminal osteogenic differentiation, thereby promoting OS growth. Further during osteosarcoma development, deletion of the p55 gene leads to an increase in Notch signaling resulting in upregulation of cyclin and osterix and inhibition of Runx2, then a decrease in Runx2 inhibits OPN expression. Nonetheless, it has been noted that higher O.P.N. levels in stromal or tumour cells increase OS' capacity to spread. In tumours, glucose transporters are overexpressed and associated with metastasis. OPN increases glucose uptake through integrin αvβ3 and promotes the hypoxia dependent expression GLUT-1 and GLUT-3(glucose transporters). Another interesting finding is that under hypoxic conditions, osteosarcomas exhibit an upregulation of OPN due to increased HIF-1a activity, which is mediated by the PI3K-AKT and MAPK signalling pathways. Furthermore, it has been observed that dysregulated expression of microRNA (miRNA) contributes to the growth and spread of cancer. Specifically, overexpression of mir-4262 suppresses OPN-mediated cell invasion, while intake of mir-4262 enhances OPN-mediated invasion of cells in OS cells. Additionally, upon the growth of a tumour size larger than 1 cm and the onset of hypoxia within the tumour cells, there is an increase in the expression of VEGF(vascularendothelialgrowthfactor), which is dependent on HIF1α. This occurs through the stimulation of the p65 subunit of NF-κB(nuclear factor-kB) by integrin-linked protein kinase B, which in turn promotes tumour angiogenesis. Upregulated expression of tumor cells derivative OPN increases metastatic property of tumor cells. Although clinical studies have highlighted higher levels of OPN expression in osteosarcoma patients, COX-2 expression and association with vascular endothelial growth factor (VEGF), yet serum OPN is not predictive of outcome in osteosarcoma patients. The results of the above studies indicate that elevated expression of OPN induces cascade of events leading to several changes in tumor metastasis mechanism.

Further research with clinical studies is needed to confirm the role of serum Osteopontin to substantiate the diagnostic efficiency in osteosarcoma which may provide insights about tumor pathogenesis, distinguishing defects and metastasis in osteosarcoma. Expression of serum OPN has the potential to become a useful biomarker for OS. However, substantial research is needed to confirm the role of serum OPN levels as a biomarker of OS. Sufficient blood samples should be collected from OS patients and healthy controls to conduct clinical trials and analyze OPN expression in normal blood samples to obtain a standard Serum osteopontin reference value for diagnosing osteosarcoma, predicting metastasis in osteosarcoma and concluding prognosis. Further studies may provide answers to the following questions: i) if increased level of serum OPN is due to elevated circulating OS cells; ii) if increase level of serum OPN correlates with OS grade, circulating OS cell count, disease-free survival and metastasis.

Additional Information

Disclosures
Conflicts of interest: In compliance with the ICMJE uniform disclosure form, all authors declare the following: Payment/services info: All authors have declared that no financial support was received from any organization for the submitted work. Financial relationships: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. Other relationships: All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

References