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Correlation Between Immuohistochemechal Expression of Osteopontin and Clinicopathological Parameters in Gastric Cancer

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Abstract—Background/Aims: *Numerous cancer types exhibit overexpression of osteopontin (OPN). On the other hand, nothing is currently known about the function of OPN expression in human cancers. In order to evaluate the relationship between OPN expression levels, cancer cell proliferative activity, and clinicopathological outcomes after gastric cancer surgery, this study was conducted.* ***Methodology****: We tested 43 cancer cases for the presence of OPN using immunohistochemistry. We also examined the cervical cancer cells' proliferation index using TUNEL labelling and an anti-MIB-1 antibody. The levels of OPN expression were used to categorise the gastric tumours into three separate categories. To identify the characteristics of cancer lesions, the TNM Classification of Malignant Tumours, Sixth Edition was used. We did this to look at how OPN expression relates to clinicopathological outcomes.* ***Results****: Immunohistochemistry was used to investigate OPN expression in gastric cancer, and the results showed cytoplasmic diffuse granular staining. Nineteen of the forty-three carcinomas tested showed elevated levels of OPN expression. Apoptotic index, invasiveness, lymphatic invasion, venous invasion, depth of invasion, and strong OPN expression were all positively correlated. The pathology findings revealed that intestinal tumours substantially expressed OPN.* ***Conclusions****: These findings suggest that OPN may play a key role in the spread and aggressiveness of gastric cancer.*

Keywords— *Osteopontin. Gastric cancer. Apoptotic index. Proliferating index.*

I. Introduction

Unfortunately, the prognosis for advanced stomach cancer is quite poor and it is still rather common. Even after curative surgery, a substantial percentage of cancer patients die from recurrence, which includes distant metastases, lymph node metastases, and carcinomatous peritonitis. Glycine, arginine, glycine, aspartate, and serine comprise the cell-binding peptide sequence of osteopontin (OPN), a phosphorylated and sialated non-collagenous acidic bone matrix glycoprotein. There are just a few of organs where OPN has been found; they include the stomach, kidney, lungs, breasts, and smooth muscles. One such cytokine that is linked to a quick response to bacterial infection that relies on T-cells is OPN. Although our understanding of OPN's role in tumour cells is limited, it has been postulated that it has cell-attaching and cell-signaling capabilities via integrin-mediated signal transduction in relation to cancer metastasis. Previous research in gastric cancer has shown a link between OPN expression and clinicopathological results. (1-2) A studies demonstrated that OPN inhibits cell death in gastric cancer cells in vitro, and another suggested that OPN might promote tumour growth and metastasis by preventing tumour cells from apoptosis.(3-4) But how exactly OPN controls stomach cancer proliferative activity is still a mystery. Accordingly, the current investigation set out to answer the question of whether or not gastric cancer OPN expression corresponds with clinicopathological features and inhibits apoptosis.

II. Materials and Methods

The study's participants were patients who had surgery in our department in 2023 or 2024 after receiving a stomach cancer diagnosis. The participants' ages ranged from 31 to 81 throughout the study, with an average age of 67.4. A 4% paraformaldehyde solution in 0.1 M PBS was used to fix tissues, both malignant and healthy, for one night at 4 degrees Celsius. They were wrapped in paraffin wax after being dehydrated using various alcohol strengths. The subsequent slices were 4 micrometres thick and underwent further immunohistochemistry and standard staining with haematoxylin and eosin. We assessed the tumor's invasion depth, lymphatic invasion, venous invasion, lymph node metastasis, and stage using the TNM Classification of Malignant Tumours, 7th Edition criteria. Furthermore, intestinal gastric cancer and diffuse gastric cancer were distinguished using the Laurens approach.(5)

Immunohistochemistry:

Monoclonal antibodies (MIB-1; DAKO Corporation, Carpinteria, California, USA) were used to screen for human OPN. To remove endogenous peroxidase activity, sections were subjected to graded ethanol dehydration, xylene deparaffinization, and a 3% hydrogen peroxide in methanol incubation for 30 minutes at room temperature. To avoid non-specific binding, we used 10% normal goat serum in PBS for 30 minutes at room temperature. The primary antibody response (OPN 1:100; MIB-1 1:50) was then allowed to settle for a full night at 4 degrees Celsius. After replacing the biotinylated goat anti-rabbit IgG secondary antibody and streptavidin-peroxidase conjugate with EnVision (DAKO Corporation), the sections were incubated for sixty minutes.To get the desired colour, samples were incubated in a solution containing 0.5% 3,3-diaminobenzidine and 0.01% hydrogen

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peroxide in a 0.05 M Tris-HCl buffer with a pH of 7.2 for two to ten minutes. Each slice was examined and photographed separately using a light microscope. The proper primary antibody was not employed since the control staining was performed using normal rabbit serum.[6]

Terminal Deoxynucleotidyl Transferase-Mediated dUTP Biotin Nick End Labeling Staining:

This study used the terminal deoxynucleotidyl transferase-mediated dUTP biotin nick end labelling technique to measure the frequency of apoptotic cells in stomach cancer. For this, the instructions for the TaKaRa In Situ Apoptosis Detection Kit (TaKaRa, Shiga, Japan) were adhered to. The deparaffinized and rehydrated sections were digested for twenty minutes at room temperature using a solution that included twenty micrograms per millilitre (μg/ml) of proteinase K, which was obtained from Sigma-Aldrich in St. Louis, Missouri, USA. The slides were cleaned with distilled water and then submerged in a solution of 2% hydrogen peroxide in distilled water for ten minutes in order to prevent the enzyme from oxidising naturally. After being cleaned in 7.4 pH PBS, the slices were incubated in equilibration buffer for ten minutes at room temperature. Concurrently, the TdT enzyme was substituted with distilled water to produce the control segments.[7]

Immunohistochemical Evaluation:

Three sections of each slide were examined for the presence of OPN in order to verify and correlate the tissue diagnosis. The criteria for classifying OPN expression was the proportion of cancer cells that had OPN labelled in their cytoplasm. The following categories were utilised for classification: weak or focused expression (±), moderate expression with a focal high expression (1+), and strong expression (2+). Two writers examined OPN expression despite without knowing the patient's medical history. In situations where alternative assessments were made, a final consensus was reached by carefully reviewing the visuals shown on the same computer screen once again.[8]

Evaluation of Apoptotic and Proliferating Cells:

To determine the ratio of apoptotic to proliferative cells, hundreds of cancer cells from each patient were counted within a predetermined region using a light microscope with an objective of ×40. The percentage of cells that were positive for proliferating or apoptotic events per 1,000 cancer cells was determined using the MIB-1 index (MI) and the apoptotic index (AI).(9)

Statistical Analysis:

Utilising a software application known as Stat View®, which was developed by SAS Institute Inc. in Cary, North Carolina, United States of America, the statistical analysis was carried out. Our investigation into the connection between the expression of OPN and the clinicopathologic characteristics of gastric cancer was conducted via the use of the $\chi 2$ test. The t-test was chosen as the selected statistical approach for the purpose of determining whether or not there was a link between the expression of OPN and either the MI or the AI. In the event when the P-value is lower than 0.05, we are able to assert that there is a statistically significant difference.(10)

TABLE 1: Clinicopathological distribution of osteoponton in gastric cancer.

Variables	OPN ±	OPN 1+	OPN 2+	*P* value
Depth of invasion				
Tis	6	5	3	<0.05
T1	2	3	2	
T2	3	1	7	
T3	1	3	4	
T4	0	0	3	
Lymph node metastasis				
N0	9	8	7	<0.05
N1	2	2	6	
N2	0	1	3	
N3	1	1	3	
Histological type Intestinal type	4	5	12	<0.05
Diffuse type	8	7	7	
Lymphatic invasion				
L0	10	7	4	<0.05
L1	2	5	15	
Venous invasion				
V1	10	11	15	<0.05
V2	2	1	4	
TNM stage				
0	6	5	3	<0.05
1A	1	2	4	
1B	2	2	3	
2	1	1	3	
3A	0	0	2	
3B	1	1	3	
4	1	1	1	

III. RESULTS

Specificity of Immunohistochemical Staining:

Unmodified mouse immunoglobulin G did not cause any noticeable response in the negative control sections. Macrophages and smooth muscle cells had relatively high levels of OPN immunoreactivity. (11)

Immunoreactivity of OPN in Normal Gastric Mucosa:

The fundic gland area was shown to have the bulk of osteopontin-positive cells in normal stomach mucosa. However, there was also a little protein expression in the cells of the neck's mucosal membrane. Additionally, OPN was detected in a limited number of cells in the mucous neck and cells around the pyloric gland.[12]

Immunoreactivity of OPN in Gastric Cancer Tissue:

Based on osteopontin immunoreactivity, gastric cancer cells had thin and rough granular immunoprecipitates in their cytoplasm (Figure 1). After cancer cells entered, the lymphatic system became extensively discoloured (Figure 2). In addition, only a tiny fraction of inflammatory cells (mostly macrophages) were OPN positive. This was decided by researchers. Macrophages in tumour stroma stained strongly with OPN. Cancer cells and macrophages in the tumor-invaded region were more OPN positive. However,

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macrophage infiltration in malignant tissue did not correlate with clinicopathological features. Test findings confirmed this.(13) Cancer cell OPN expression correlated with lymph node metastases, histological type, lymphatic invasion, venous invasion, stage grouping, and invasion degree.

Apoptotic and MIB-1 Indices:

AI revealed cancer cell scores of 11.3±2.9, 9.1±3.1, and 6.1±2.4 for the ±, 1+, and 2+ groups, respectively. Table 2 shows a substantial difference (P<0.05) between the 1+ and 2+ groups, as well as the ± and 1+ groups. From Table 3, we learn: The mean cytotoxicity (MI) of cancer cells in the ±, 1+, and 2+ groups was 15.4±4.9, 24.5±6.6, and 34.9±7.8. The statistical analysis shows a substantial difference (P<0.05) between the 1+, 2+, and ± groups. Statistical study showed a significant negative correlation between stomach cancer, myocardial infarction, and AI (P<0.05).

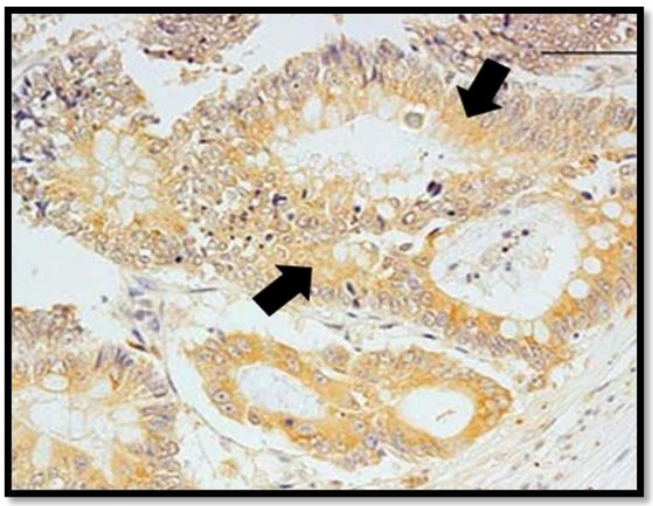

Figure 1: In malignant tissue, osteopontin exhibits immunoreactivity. At a magnification of 40, the cytoplasm of cancer cells showed fine and rough granular immunoreactivity.

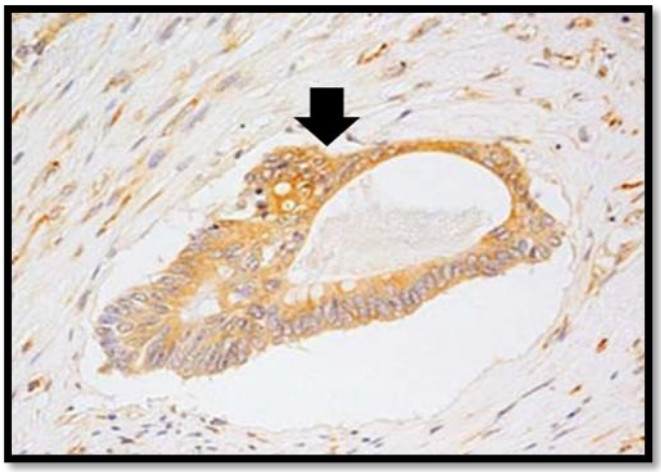

Figure 2: At a magnification of 40x, lymphatic invasive carcinoma cells showed a strong immunoreactivity to OPN.

TABLE 2: Osteopontin (*OPN*) expression in gastric cancer and apoptotic index (*AI*).

OPN Level (X-Axis)	Estimated AI Value (Mean)	Observations
+/-	11.3±2.9	Highest AI value recorded.
+	9.1±3.1	Moderate decrease in AI.
++	6.1±2.4	Lowest AI value recorded

TABLE 3: Osteopontin (OPN) expression in gastric cancer and proliferating index (PI).

OPN Level (X-Axis)	Estimated PI Value (Mean)	Observations
+/-	15.4±4.9	Lowest PI value recorded.
+	24.5± 6.6	Significant increase in PI.
++	34.9±7.8	Highest PI value recorde

IV. Discussion

The calcium-binding phosphoprotein osteopontin may be needed for multiple apparently unrelated biological activities. Human and animal studies have linked OPN expression to cancer development and metastasis. OPN was found in stomach, duodenum, colon, and esophageal cancer cells and macrophages in a recent investigation. OPN mRNA was only found in macrophages. The oxidative protein matrix (OPN) produced by macrophages and the alpha v beta three integrin may help cancer cells adhere together. Macrophages were the only ones with OPN mRNA during tumour invasion. This supports the idea that macrophage-produced OPN affects cancer cell adhesion, metastasis, and invasion. However, few studies have examined the link between OPN expression and stomach cancer clinicopathology. OPN protein expression was associated with age, tumour depth, histological grade, and hemogenous metastases in one study. In contrast, lymph node metastases seemed unrelated. This sequence shows how much stomach cancer has infiltrated lymph nodes, distant metastases, and OPN expression. This applies globally and regionally. Immunohistochemical labelling was used to examine OPN expression in 43 surgically excised stomach carcinomas. We investigated whether OPN expression correlated with stomach cancer pathology, AI, and MI. Nearly 50% of 43 people had a significant immunological response to OPN. OPN antibody levels were connected to blood vessel invasion, lymph node depth, metastases, invasion, and the final stage. After entering the lymphatic system, stomach cancer cells displayed high proliferative, low apoptotic, and high OPN immunoreactivity. Through lymphatic tubes, it occurred.15. New study contradicts MI-AI conclusions. AI was strongly linked to stomach cancer myocardial infarction, while other studies found no connection (16–17). In addition to collagen, bone morphogenetic protein (OST) may interact with cell surface receptors. These integrins—alpha v beta 3, 1, and 5—bind OPN. The arginine-glycine-aspartic acid OPN surface receptor is perhaps the best-studied. This happens because OPN receptors are alpha v beta 3. The OPN receptor protein also has multiple CD44 variations, according to study. Studies led to this finding. OPN interacts with cell surface receptors early in the signalling cascade that causes cell proliferation and death. Every one of them might come from these interactions.GM-CSF, OPN, and CD44 have been demonstrated to increase the proliferation of IL-3-dependent

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murine bone marrow cells and the proB cell line Ba/F3. Proof comes from these three pieces cooperating. Additionally, research indicates that oxyphloropoietin (OPN) on endothelial cells interacts with alpha v beta three integrins to activate the NF-κB pathway and prevent cell death. Gastric cancer may have links between apoptotic responses and cell proliferation or surface receptors such CD44 and alpha v beta three integrins. These receptors include CD44. These results suggest that stomach cancer cells' OPN production may contribute to metastasis. The present research shows a robust association between OPN expression and low AI and high MI in stomach cancer, supporting this idea. Statistically significant correlation discovered. [19] found a considerable negative connection between MI and AI. This research suggests osteopontin may be a cancer treatment target. OPN expression may decrease at transcriptional and RNA message levels. Synthetic peptides and antibodies may inhibit OPN protein synthesis. OPN receptor attack is another possibility. Small molecule inhibitors targeting integrin alpha v beta 3 are being studied as alternatives to medicines. CD44 is used therapeutically for cytotoxic and immunological objectives. These two therapeutic objectives are widely used. Cancer prevention may include OPN targeting, which slows cell proliferation and promotes death. This research supports this potential therapy. By evaluating OPN expression in endoscopically obtained preoperative tumour tissue samples, therapy may be tailored.(20).

V. Conclusion

Researchers looked at how osteopontin reacted with stomach cancer cells. There was a correlation between OPN expression and lymphatic invasion, lymph node metastases, invasion depth, and disease stage. One possible approach to improving apoptosis and decreasing gastric cancer cell growth is to suppress osteopontin (OPN), as OPN production may decrease apoptosis and increase proliferation.

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