

## Osteopontin Expression and its Potential Role as Prognostic Factor and Therapeutic Target

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### Abstract

A correlation between the over expression of Osteopontin (OPN), E-cadherin,  $\beta$ -catenin, and cyclooxygenase 2 and poor prognosis has been previously suggested in solid tumors. The aim of the ITACA-S translational study was to further investigate the correlation of these immunohistochemical biomarkers with the outcome of radically resected gastric cancer patient. The immunohistochemistry expression of osteopontin, E-cadherin,  $\beta$ -catenin, and cyclooxygenase 2 was detected in 346 primary gastric tumor tissue samples from patients enrolled in the ITACA-S trial which randomized patients to receive adjuvant chemotherapy with either 5-fluorouracil and leucovorin or a sequential regimen of infusional 5-fluorouracil and leucovorin plus irinotecan followed by cisplatin and docetaxel. A higher expression of OPN was associated to diffuse histotype, high grade and peritoneal relapse, but not with TNM stage. It was associated with higher risk of recurrence and metastases. Furthermore, OPN overexpression was identified as an independent prognostic factor for both relapse-free and overall survival. The abnormal expression of E-cadherin or  $\beta$ -catenin was correlated with more advanced disease stage and a poor outcome.

**Keywords:** Gastric cancer; Metastatic disease; Osteopontin

### Background

Gastric Cancer (GC) represents the fifth most common malignancy in the world and even though its incidence has significantly decreased world-wide since 1970s, GC is still the third cause of cancer-related death, accounting for 107,000 death in Europe [1].

Furthermore, despite all the progress globally made, the 5-year Overall Survival (OS) rate of GC is about 20%, with worse prognosis for metastatic disease. In fact, the median OS for metastatic GC is approximately one year, even when patients are treated with chemotherapy [2].

The most recent results on the molecular and biological characterization have disclosed the heterogeneity of this disease. Both risk and prognostic factors have been investigated in the past decades in order to improve the therapeutic landscape; however, more information is needed. Osteopontin (OPN) is a matrix extracellular phosphorylated glycoprotein with multifaceted roles in the interaction between cancer cells and the tumor microenvironment. OPN signalling results in various functions, including prevention of apoptosis, modulation of angiogenesis, malfunction of Tumor-Associated Macrophages (TAMs) and degradation of extracellular matrix, which lead to tumor formation and progression [3]. In addition to OPN, E-cadherin is another adhesion protein that is also crucial in the maintenance of cell polarity and differentiation and in the suppression of cancer invasion. Moreover, the E-cadherin-  $\beta$ -catenin adhesion complexes have a key role in anchorage mechanisms, and  $\beta$ -catenin is involved in the Wingless/Wnt signaling pathway [4].

Different studies also measured the expression of OPN in primary tumor tissue in GC patients and investigated the clinical relevance of

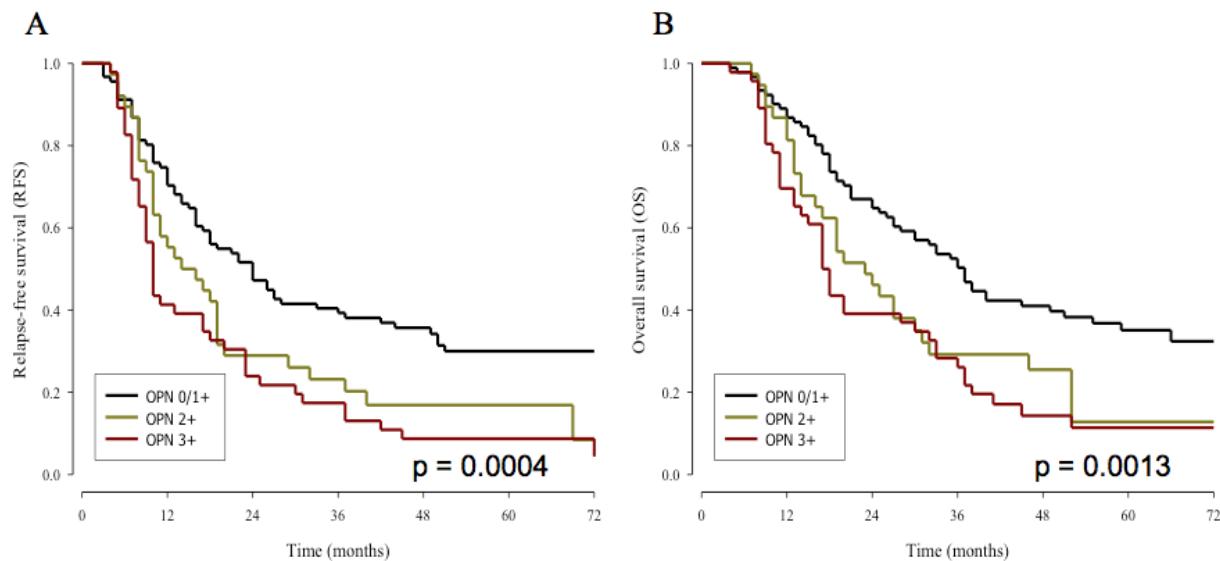
the potential relationship between OPN and survival outcomes. The results of these studies were, however, controversial and inconclusive.

### Study

In light of such considerations, we designed a translational study aiming to analyze the Immunohistochemistry (IHC) expression of OPN, E-cadherin,  $\beta$ -catenin, and cyclooxygenase-2 (COX-2) in 346 primary gastric tumor tissue samples from radically resected patients enrolled in the ITACA-S trial [5]. This was a phase III study randomizing patients to receive adjuvant chemotherapy with either monotherapy with 5-fluorouracil and leucovorin or a sequential regimen of infusional 5-fluorouracil and leucovorin plus irinotecan followed by cisplatin and docetaxel (FOLFIRI  $\rightarrow$  CDDP+TXT). The results of the clinical study documented no difference in terms of OS and Disease Free Survival (DFS) between the two groups. In this homogenous population, we investigated the potential prognostic role of OPN, E-cadherin,  $\beta$ -catenin, and COX-2 over-expression.

Tumor specimens from 346 patients were analyzed. The immunohistochemical staining for OPN, E-cadherin,  $\beta$ -catenin, and COX-2, done with an EnVision® FLEX+detection system, was conducted by two qualified pathologists, blind to any patients' history. No patient was lost to follow-up, the median of which was 61 months (IQR, 48-75 months).

Our results showed that highly expressed OPN was associated with diffuse type, poorly differentiated tumor but not with TNM stage. In addition, the overexpression of OPN was an independent prognostic factor for both relapse-free survival (RFS) and OS (Figure 1).



**Figure 1:** Kaplan-Meier curves for relapse-free survival (A) and overall survival (B) according to the osteopontin (OPN) expression groups.

In particular the 6-year RFS was 49.7% (95% CI, 42.7-57.9%) in OPN 0/1+ vs. 34.0% (95% CI, 23.3-49.5%) in OPN 2+ vs. 22.9% (95% CI, 14.1-37.1%) in OPN 3+ subgroup. The 6-year OS was 53.0% (95% CI, 45.8-61.4%) in OPN 0/1+ vs. 43.2% (95% CI, 32.7-57.2%) in OPN 2+ vs. 34.2% (95% CI, 24.7-47.5%) in OPN 3+. Moreover, the expression of E-cadherin was significantly associated with RFS and OS ( $p=0.003$  and  $0.001$ ). The 6-year RFS was 47.1% (95% CI, 40.9-54.1%) for normal expression vs. 22.8% (95% CI, 14.1-37.2%) for abnormal one. The 6-year OS was 51.0% (95% CI, 44.5-58.4%) and 37.5% (95% CI, 29.0-48.3%), respectively.

The abnormal expression of b-catenin was significantly correlated with OS, with 6-year OS rates of 52.0 % (95% CI, 44.2-61.2%) for patients with normal expression versus 42.4 % (95% CI, 35.5-50.7%) with abnormal one. The correlation with the RFS was not statistically significant. Furthermore, the analyses regarding the abnormal expression of COX-2 were non-significant.

A subgroup analysis revealed that 3+OPN expression was not significantly associated with patient RFS or OS for stage IB/IIIA disease ( $p=0.476$  for RFS and  $p=0.774$  for OS). On the contrary, a statistically significant effect on OS was observed in patients with stage IIIB/IIIC GC. In particular, the 6-year OS rate was 32.4% in the 0/1+OPN expression group, 12.8% in 2+OPN expression group and 11.4% in the 3+ OPN expression group ( $p=0.001$ ). The 6-year RFS rates were similar.

We also speculated on the effect of monotherapy with fluoropyrimidine compared to sequential regimen (FOLFIRI → CDDP +TXT) according to OPN expression. In the OPN 0/1/2+group no major differences were observed in the RFS and OS curves while in the OPN3 group the 6-year RFS was 12.4% (CI, 4.8-31.8%) in the fluoropyrimidine arm vs. 36.7% (CI, 23.4-57.4%) in the sequential chemotherapy arm ( $p=0.075$ ), whereas 6-year OS estimated were 22.8% (CI, 12.1-43.1%) vs. 45.0% (CI, 31.1-65.1%).

The peculiarity of the present study consists in the analysis of the potential prognostic impact of pathologic factors within the context of a clinical trial in a homogeneous patient's population.

Finally our data showed that the poorer outcome associated with OPN overexpression was maintained only in the subset of patients with more advanced disease stage, i.e. IIIB-IIIC. In fact, OPN may drive the development of a particularly aggressive phenotype that is acquired only in presence of other permissive factors emerging at later stages of GC progression. These data confirm that the expression of OPN may have a prognostic role especially in the advanced disease as suggested also by Cao et al. [6] However, all these observations derive from non-prespecified subgroup analyses and, although intriguing, should be considered carefully as hypothesis generating. In particular, intensification of adjuvant treatment could be investigated prospectively in the high-risk subgroup of patients with more advanced disease stage radically resected and OPN overexpression.

## Implications

In the last decade, emerging evidence has refined the key role played by OPN as a biomarker and a potential target for cancer therapy [6]. This is due to the fact that OPN is a secretory extracellular matrix (ECM) protein that is involved in a series of physiopathological processes including cell adhesion, migration, invasion, proliferation and even inflammation [7-9]. Its altered expression has been reported in different tumor types such as breast cancer, prostate cancer, non-small cell lung cancer, colorectal cancer, glioma and hepatocellular carcinoma [10-15]. OPN expression is significantly elevated in both primary GC and metastatic lesions, and it is absent in negatively gastric mucosa. Furthermore, in GC, OPN expression is significantly associated with clinicopathological parameters such as proliferative index, stage disease, lymph nodes and vascular invasion and distant metastasis. In resected GC we documented that OPN expression 3+ was significantly associated with poorly differentiated and diffuse-type tumors but not with TNM stage.

Regarding the prognostic role, several studies have been published on the potential correlation between OPN expression in tumor tissue and survival; unfortunately, the results have been controversial and inconclusive so far. Recently Gu et al. have performed a meta-analysis to further investigate the potential prognostic role of OPN in resected GC patients [16]. They analyzed a total of ten studies involving 1775 patients [17-25]: nine were Asian studies and one was the Italian one performed by our group. Two were the methods used to detect OPN expression: IHC in eight studies and ELISA on plasma specimens in two studies. High OPN expression was found to be correlated with poor OS (HR=1.59, 95% CI: 1.15-2.22, p=0.006) in the pooled analyses. Subgroup analyses were conducted proving the OPN prognostic value for Asian patients (HR=1.64, 95% CI=1.11-2.41, p=0.012) and for patients receiving surgical resection (HR=1.6, 95% CI =1.04-2.48, p=0.034). Regarding the detection method, OPN overexpression remained a prognostic marker when detected via ELISA method. When tested by IHC, OPN expression was also associated with poor OS, although it did not show any statistical significance.

The results of our study are in line with the data obtained by this recent meta-analysis. Our results strongly support that the use of OPN as a new biomarker may help improve the outcome prediction and drive the decision making process for the treatment of patients undergoing radical GC resection.

Additionally, the role of OPN as a therapeutic target for tumor treatment has been largely investigated. Potential strategies use an OPN antibody to block the binding of OPN to its receptors; this binding would ultimately block the signal downstream and deliver, to tumor cells, the small interfering RNA (siRNA) targeting OPN with the final aim to decrease directly the expression of OPN itself and to annul the effect triggered by OPN overexpression [6].

In vitro and in vivo data from Tang et al. [26] also showed that OPN may inhibit tumor growth and GC cells migration via RNA interference (RNAi).

Furthermore, in 2011, Wang et al. silenced the expression of OPN in GC cell line SGC7901 via a lentiviral-OPN siRNA technology. Their data showed a longer survival time in those mice implanted with OPN-SGC7901 cells [27]. However, the expression of microRNA (miRNA) might be extremely heterogeneous due to several factors as hypoxia and inflammation in tumor microenvironment. This heterogeneity represents a concrete obstacle in identifying the specific target for miRNA. The design of an adequate delivery vehicle for miRNA represents another big challenge since it has to have high stability and sensitivity in vivo and lower toxicity.

These results represent the rationale for further investigation on the OPN network and its potential use as a therapeutic target.

At present, however, no clinical trial targeting OPN is in progress for tumor treatment. This is mainly due to the fact that OPN is an important cytokine that also mediate normal physiological functions; therefore its therapeutic blocking might result in severe adverse events. Further investigations are warranted to develop better therapeutics strategies.

In conclusion, OPN expression seems to have a greater potential as prognostic marker.

Our study represents the first step towards the discovery of prognostic and predictive biomarkers and of novel treatments focused

on newer agents targeting the molecular drivers of the neoplastic progression.

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