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Original Article

Osteopontin expression is associated with progression and adverse prognosis in patients with resectable gastrointestinal stromal tumor

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Abstract: Objectives: Osteopontin (OPN) is reported to be particularly associated with the progression of several human malignancies. This study was designed to examine the clinicopathologic significance of OPN in gastrointestinal stromal tumor (GISTs). Methods: The level of OPN expression in a large cohort of resectable GISTs was evaluated with immunohistochemistry. Its correlation with the clinicopathologic parameters of patients with resectable GISTs was analyzed. A survival analysis was performed to evaluate the prognostic significance of OPN expression using the Kaplan-Meier method. Results: In 108 patients with resectable GISTs, the most high-risk GISTs had a strong level of OPN expression. Strong OPN expression was also significantly associated with tumor size, mitosis, and recurrence, but not gender and age. Patients with weak OPN expression had a relatively longer disease-free survival compared to patients with strong OPN expression. Conclusions: OPN expression is a putative marker for tumor progression and an adverse prognosis in GISTs.

Keywords: Osteopontin, gastrointestinal stromal tumors, immunohistochemistry, prognosis

Introduction

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the alimentary tract and originate from interstitial cells of Cajal or their precursors [1]. Gain of function mutations in c-KIT or platelet-derived growth factor receptor α (PDGFRα) are observed most frequently and contribute to tumor cell proliferation and survival [2]. Complete surgical resection (R0 resection) is still the mainstay of the treatment for GISTs. The 5-year survival rate ranges from 35% to 65% in patients with primary resectable GISTs, depending on the risk grade of the tumor [3]. The median recurrence time is approximately 12-16 months [4]. Recent studies have confirmed that imatinib, a tyrosine kinase inhibitor, has a remarkable effect on the treatment of GISTs, resulting in an improved prognosis [5, 6]. Exploring predictors associated with GIST progression and prognosis has become increasingly important and beneficial to understanding their aggressive behaviors.

Osteopontin (OPN) is a phosphorylated and glycosylated secretory protein of the extracellular matrix that can be expressed in miscellaneous cell types [7]. OPN contributes to various physiologic and pathologic behaviors, such as bone remodeling, angiogenesis, wound healing and inflammatory cell accumulation [8, 9]. Several studies have also documented that increased OPN expression contributes to the aggressive behaviors of tumor cells, which is also useful as a biomarker for poor prognosis of malignancies [10-12]. However, the role of OPN in GISTs is seldom investigated. In this study, we examined the expression level of OPN in resectable GIST specimens and evaluated the relationship between OPN expression and the clinical parameters and prognosis of GIST patients.

Materials and methods

Patients and specimens

Between January 2012 and December 2012, 108 patients who received complete surgical

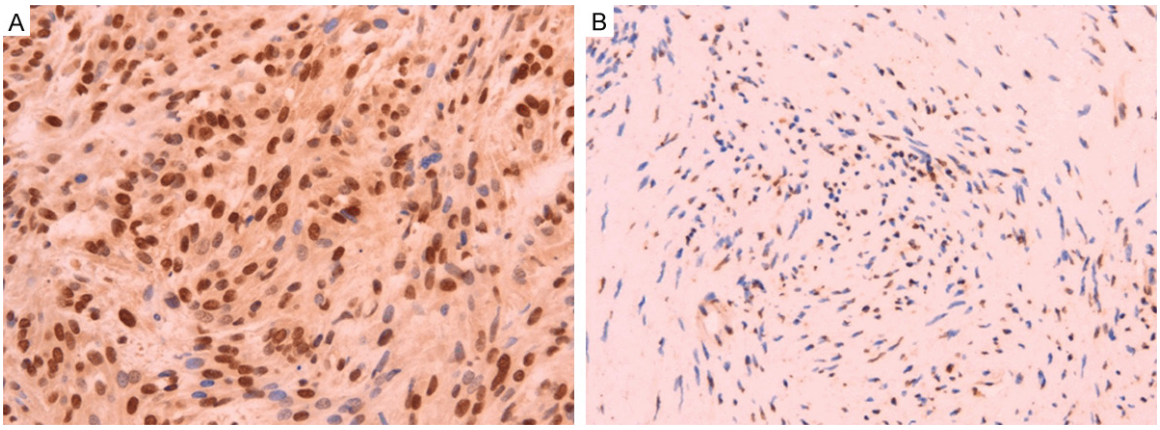

Figure 1. Representative immunohistochemical staining of OPN in resectable GISTs. Positive staining for OPN was defined as brown-yellow cytoplasmic staining. An immunohistochemical staining score of more than 3 was considered strong expression (A), and a score less than 3 was considered weak expression (B). Original magnification (× 400) (Axiostar-plus, Carl Zeiss microimaging, Germany).

treatment for primary GISTs in the First Affiliated Hospital to Zhejiang University were enrolled in this study. Informed consent was obtained from all patients, and the Ethics Committee of the Hospital approved this study. The diagnosis of GISTs was pathologically and clinically proven. To eliminate possible interference factors, we excluded all cases that met any one of these criteria: resections with positive margins, adjuvant imatinib treatment, a family history of GISTs, and a history of other malignancies. Demographic data and pathologic stage were collected. GISTs were categorized into different grades according to the National Institutes of Health (NIH) Consensus Criteria for GIST risk stratification: very-low-risk, low-risk, intermediate-risk and high-risk [13]. Patients were regularly followed at our outpatient department with abdominal computed tomography (CT) every 3 months or 6 months for the first 3 years after surgery depending on high-risk and non-high-risk grade, respectively. The follow-up thereafter for all patients was every 6 months.

Immunohistochemistry

Formalin-fixed and paraffin-embedded blocks from 108 GIST specimens were prepared for immunohistochemical staining according to previously described procedures [14]. Briefly, serial 4 μm thick sections were routinely dewaxed, dehydrated and pressure-cooked for antigen retrieval. The sections were then incubated overnight at 4°C with primary antibody. Antihuman OPN rabbit polyclonal antibody (Sigma) was diluted 1:100 in PBS with 1% bovine serum albumin (BSA). The sections were counterstained with hematoxylin, dehydrated and mounted. A tissue section of human pancreatic adenocarcinoma was used as a positive control. The negative control was created by substituting the OPN primary antibody with PBS containing 1% BSA.

Scoring criteria

Two specialized pathologists evaluated and graded the degree of immunohistochemical staining independently. Consensus was reached through rescoring when there were grading discrepancies. Positive OPN staining was defined as brown-yellow cytoplasmic staining. Semiquantitative evaluation was performed to establish the grade of immunohistochemical staining. For each section, five adjacent fields at a magnification of × 400 were observed using light microscopy (**Figure 1**). The staining intensity was scored as negative (0), weak (1), moderate (2) and strong (3). The percentage of positive staining cells was scored as ≤ 5% (0), 6%-25% (1), 26%-50% (2), 51%-75% (3) and > 75% (4). The terminal score of each field was determined by adding together the staining intensity and the percentage of positive staining cells. A terminal score of 3 or less was considered weak expression. An immunohistochemical staining score greater than 3 was considered strong expression.

Statistical analysis

All statistical analyses were performed using the SPSS 22.0 package. Descriptive data are expressed as mean ± SD. Categorical variables

Table 1. Demographics and clinicopathologic characteristics in patients with resectable GIST based on OPN expression

Characteristic	Total	OPN expression		*P* value
		Weak ≤ 3	Strong > 3	
Age (yr)				0.365
< 63	49	30 (61.2%)	19 (38.8%)	
≥ 63	59	31 (52.5%)	28 (47.5%)	
Gender				
Male	59	33 (55.9%)	26 (44.1%)	0.899
Female	49	28 (57.1%)	21 (42.9%)	
Tumor size (cm) (mean ± SD)	4.86 ± 3.58	4.07 ± 2.80	5.88 ± 4.21	0.013
Mitosis (HPF) (mean ± SD)	5.62 ± 4.25	4.07 ± 1.70	7.64 ± 5.57	< 0.001
Risk group				0.066
Very Low	17	11 (64.7%)	6 (35.3%)	
Low	43	29 (67.4%)	14 (32.6%)	
Intermediate	14	8 (57.1%)	6 (42.9%)	
High	34	13 (38.2%)	21 (61.8%)	
Recurrence				< 0.001
Yes	20	2 (10.0%)	18 (90.0%)	
No	81	52 (64.2%)	29 (35.8%)	

OPN, osteopontin; SD, standard deviation; HPF, high-power fields.

were compared between groups using the χ^2 test, while continuous variables were compared with an independent sample *t* test. A survival analysis was computed with the Kaplan-Meier method, and disease-free survival (DFS) was compared using the log-rank test. The Cox proportional hazard model was applied to the multivariate analysis. A probability value of less than 0.05 ($P < 0.05$) was considered statistically significant.

Results

Demographic and clinicopathologic characteristics of patients with resectable GISTs

We collected 108 patients who underwent complete surgical resections without adjuvant imatinib. The cohort included 49 women and 59 men with a mean age of 62.4 ± 10.5 years (median: 63 years, range: 34 years to 83 years). Maximum tumor diameter varied from 0.6 cm to 16.5 cm (median = 3.6 cm), and mitotic counts varied from 1/50 HPFs to 25/HPF (median = 5/50 HPFs). According to the NIH Consensus Criteria, GIST patients were categorized into very-low-risk (n = 17), low-risk (n = 43), intermediate-risk (n = 14) and high-risk (n = 34) groups.

Clinicopathologic significance of OPN expression in patients with resectable GISTs

We evaluated the relative levels of OPN expression in GIST specimens using immunohistochemistry (**Figure 1**). Patients with strong OPN expression had significantly larger tumor sizes and increased mitoses ($P = 0.013$ and $P < 0.001$, respectively). However, there was no significant difference between risk status and OPN expression ($\chi^2 = 7.17$, $P = 0.066$). There were also no significant differences in OPN expression between different gender and age groups ($\chi^2 = 0.016$, $P = 0.899$ and $\chi^2 = 0.821$, $P = 0.365$, respectively). Notably, strong OPN expression was clearly related to an increased recurrence rate of resectable GISTs ($\chi^2 = 6.72$, $P = 0.01$). Results are summarized in **Table 1**.

The results showed that there was a predominance of strong OPN expression in patients with high-risk GISTs (61.8%), despite no significant difference between risk groups as defined by the NIH Consensus Criteria. We further compared the OPN expression between high-risk and non-high-risk (including the very-low-risk, low-risk and intermediate-risk) GISTs, and the results were significantly different ($\chi^2 = 6.72$, $P = 0.01$).

Survival analysis of OPN expression in patients with resectable GISTs

Of 108 GIST patients, 7 were lost to follow-up, and 101 were followed at the time of this study (range: 9-66 months). The impact of several variables, such as OPN expression, risk grade, gender, and age on DFS were calculated. Based on our univariate analysis, GISTs with strong OPN expression had decreased DFS compared to that of GISTs with weak OPN expression. (50.064 ± 3.184 months vs 64.885 ± 0.824

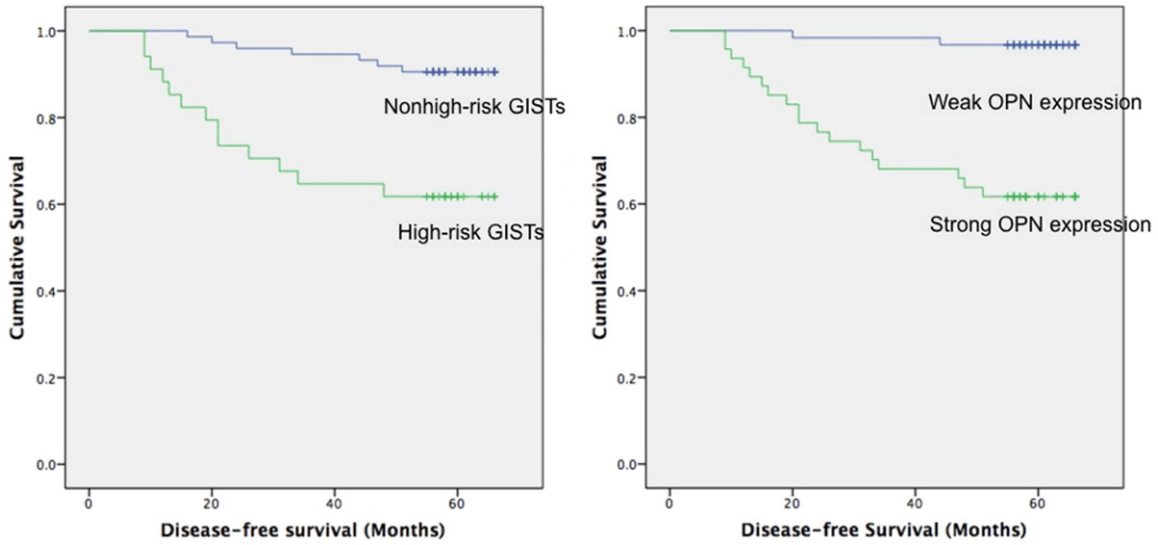

Figure 2. Survival analysis using the Kaplan-Meier method. High-risk grade and strong OPN expression suggest a poor prognosis in patients with resectable GISTs.

months, respectively. P < 0.001) (**Figure 2**). High-risk GISTs had a DFS disadvantage (48.647 ± 3.963 months) compared to that of nonhigh-risk GISTs (62.932 ± 1.197 months) (P = 0.001) (**Figure 2**). The multivariate analysis further demonstrated that strong OPN expression (P = 0.001) and high-risk disease (P = 0.007) were independent predictors of an adverse prognosis in patients with resectable GISTs.

Discussion

GISTs are the main cohort of neoplasms that originate from the mesenchymal tissues of the digestive tract. The clinical manifestations of GISTs are nonspecific and cover a broad spectrum of clinical presentations. In the past, GISTs were frequently misdiagnosed as leiomyomas, leiomyosarcomas, leiomyoblastomas, schwannomas, and so on [15]. Until the discovery and affirmation of the c-KIT and PDGFRα genetic mutations in 1983, GISTs were not an independent entity and family [16]. Over several years, advancements in genetic and immunohistochemical features have led to advancements in the diagnosis and therapy of GISTs. Most GISTs (approximately 95%) are positive for CD117, which is the main diagnostic biomarker [15]. DOG-1 positive staining is another significant immunohistochemical biomarker that can help diagnose GISTs in cases with negative CD117 [17]. Imatinib administration has significantly improved the prognosis of patients with advanced or unresectable GISTs [18, 19]. It is valuable to explore correlations between potential biomarkers and GIST diagnosis and prognosis.

Here, we conducted the largest study thus far to assess the role of OPN in GISTs by analyzing the OPN expression levels of 108 resected GIST specimens, which were categorized into different risk statuses according to the NIH Consensus Criteria. Our immunohistochemical findings showed that high-risk GISTs had a relatively stronger OPN expression compared to non-high-risk GISTs. We did not find any significant differences in OPN expression between different age and gender groups. These results suggest that OPN has a tumor-promoting role in the progression of GISTs. To investigate the potential of OPN as a prognostic marker, we conducted a regular follow-up for these patients with resectable GISTs. We found that OPN expression was positively correlated with recurrence rate. Our survival analysis further suggested that patients with weak OPN expression had a relatively longer DFS compared to control group with strong OPN expression. Other possible factors associated with DFS include risk status, gender and age. The findings of our multivariate analysis showed that strong OPN expression and high-risk status are independent predictors of an adverse prognosis.

It is well established that OPN plays a very important role in malignant transformation and contributes to the progression of most human

malignancies, such as endometrial cancer, prostate cancer, lung cancer, and colorectal cancer [20-22]. OPN was also reported to be a prognostic biomarker for multiple adenocarcinomas [23-25]. Our study's results established a role of OPN in GISTs, which was consistent with the earlier reports mentioned above, regardless of the mesenchymal origin of GISTs.

In the clinic, monitoring postoperative GIST patients for recurrence has solely relied on imaging, which is not convenient or economic. No special tumor markers can serve in this duty in the way that CEA and AFP are valuable in the postoperative monitoring of colorectal cancer and hepatic cancer, respectively. Additionally, imatinib is often preoperatively administered to make certain unresectable GISTs better suitable for R0 resection. How can one confirm the best time of resection and manage the duration of imatinib? This is always difficult using imaging alone. Poruk and Takenaka reported that the serum level of OPN is an independent and useful predictor for an accurate prognosis of pancreatic adenocarcinoma and nonsmall-cell lung cancer [26, 27]. However, the significance of the serum OPN level in GISTs is undefined. By confirming the role of OPN in GIST progression and adverse prognosis, OPN as a kind of secretory protein might be a potential candidate for monitoring tumor progression and recurrence.

In conclusion, the present study identified that strong OPN expression was consistent with GIST progression and that OPN was an independent predictor of an adverse prognosis of patients with resectable GISTs. In the era of imatinib, whether OPN is a valuable biomarker for the progression and prognosis of GISTs has not been investigated. We did not enroll patients who had received imatinib administration. Further investigation on relationship between OPN expression and imatinib treatment would contribute to a much better recognition of the diagnostic and prognostic values of OPN.

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Disclosure of conflict of interest

None.

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