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ORIGINAL PAPER

Expression and Clinical Significance of Focal Adhesion Kinase in the Two Distinct Histological Types, Intestinal and Diffuse, of Human Gastric Adenocarcinoma

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Abstract Focal adhesion kinase (FAK), a non-receptor tyrosine kinase protein, acts as an early modulator of integrin signaling cascade, regulating basic cellular functions. In transformed cells, unopposed FAK signaling has been considered to promote tumor growth, progression and metastasis. The aim of this study was to assess the clinical significance of FAK expression in the two distinct histological types of human gastric neoplasia. FAK expression was assessed immunohistochemically in tumoral samples of 66 gastric adenocarcinoma cases, 30 intestinal and 36 diffuse type, and was statistically analyzed in relation to various clinicopathological characteristics, tumor proliferative capacity and patients' survival. In intestinal type carcinomas, enhanced FAK expression was significantly associated with increased tumor proliferative capacity (P=0.012). In diffuse type carcinomas, FAK staining intensity was significantly correlated with tumor size (P=0.026) and disease stage (P=0.024), presenting also a borderline association with nodal status (P=0.053). In diffuse type carcinomas, enhanced FAK expression was significantly associated with longer overall survival times (log-rank test, P=0.014), being also identified as an independent prognostic factor in multivariate analysis (Cox regression, P=0.016). In contrast, patients with intestinal type tumors and enhanced FAK expression were characterized by shorter overall survival times, without though reaching statistical significance (log-rank test, P=0.092). The current data support evidence that FAK protein may be considered as a diagnostic and prognostic marker in gastric neoplasia. Further studies conducted on larger clinical samples and highlighting on the distinct impact of the two histological types are warranted to delineate the clinical significance of FAK protein in gastric neoplasia.

Keywords Clinicopathological parameters · Diffuse · Focal adhesion kinase · Gastric cancer · Immunohistochemistry · Intestinal · Patients' survival

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Introduction

Focal adhesion kinase (FAK) is a 125 kDa cytoplasmic non-receptor tyrosine kinase enzyme initially described as a putative substrate for the Rous sarcoma virus-encoded oncoprotein $pp60^{v\text{-}src}$ [1, 2]. FAK was reported to be tyrosine-phosphorylated in response to integrin-mediated cell adhesion, integrin clustering, cell motility and migration [3, 4]. It was also shown that FAK forming a signaling complex with Src, a member of the Src family of cytoplasmic tyrosine kinases, activates downstream enzymes such as mitogen-activated protein (MAP) kinases [1, 4, 5], resulting in activation of tumor cells. Structure and functional analysis of FAK led to the identification of multiple binding interacting sites of this molecule with other proteins important for signaling. Activation and

subsequent autophosphorylation of FAK, in response to cell adhesion, leads to its association with several signaling molecules triggering signal transduction [6]. Tyrosine kinase inhibitors have also been reported to reduce tyrosine phosphorylation of FAK and subsequently decreased cellular migration of tumor cells *in vitro* [7].

FAK has been held responsible for cancer cells' uninhibited proliferation, protection from apoptosis, invasion, migration, adhesion and spreading, as well as tumor angiogenesis [8]. Direct FAK targeting resulted in the inhibition of cancer cells' malignant phenotype, while increased cancer cells' apoptotic rates either used alone or in combination with conventional chemotherapeutic agents, radiotherapy or hormonal therapy [9]. Several studies have also indicated that FAK participates in the mechanism of action of several cytotoxic substances, rendering FAK signaling as a potential target in blocking cytotoxicity [10]. In tumoral cells, FAK levels represented a higher degree of overexpression than other tyrosine kinases expressed in cancer, such as Src [11], being associated with the process of tumor invasion and metastasis [12]. *In situ*, increased FAK expression has been reported in various malignant tumors compared to normal tissue as recently reviewed by Chatzizacharias et al. [8]. However, at present, there is non extensive data available regarding its relation to clinicopathological parameters and patients' survival amongst the different types of cancer [8, 13, 14]. Overall, the clinical impact of FAK expression on patients' management and outcome seems to be controversial amongst the different types of cancer. However, there is also promising evidence that FAK expression could be considered as a prognostic factor in liver, lung and cervical neoplasia [15–18].

Gastric cancer is the second largest cause of cancer-related death worldwide, presenting the higher incidence in Japan and China, lower in Europe and the lowest in the USA [19, 20]. Studies on the development of gastric cancer suggest that genetic predisposition, infection, and diet are part of a complex interaction [20, 21]. In this context, according to an early study, FAK was expressed in only half of the 10 gastric carcinoma cases [22]. A more recent study revealed that of the 75 gastric carcinoma cases, 43 (56%) showed moderate or intense FAK immunoreactivity [23]. Significant associations were found between enhanced FAK expression and poor differentiation, deep invasion, and lymph node metastasis of gastric carcinoma; however, no other information about its diagnostic and prognostic relevance was provided [23]. Thus, a more comprehensive evaluation with respect to the clinical significance of FAK expression in gastric neoplasia is still recommended.

Moreover, it should be noted that the two different histological entities reported in gastric cancer, defined as diffuse and intestinal type are considered to be characterized by distinct behavior and genetics [24–26]. Multiple genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes and genetic instability, as well as telomerase activation are implicated in the multi-step process of human gastric neoplasia [24–26]. However, particular combinations of these alterations differ in the two histological types of gastric cancer, indicating that intestinal and diffuse carcinomas present distinct tumorogenic pathways [24–26]. In this context, there is no available data so far evaluating FAK expression separately in each histological type.

The present study aimed to assess the immunohistochemical expression of FAK protein in gastric neoplasia. Sixty-six gastric cancer cases, classified as of intestinal in 30 and of diffuse in 36, were analyzed separately to evaluate the association of FAK expression and staining intensity with clinicopathological parameters, tumor proliferative capacity, and patients' survival within the two distinct histological types.

Materials and Methods

Patients

Sixty-six gastric tumoral samples obtained from equal number of patients who underwent surgical resection due to gastric cancer were included in this study. Forty-seven of the patients were men (71%) and 19 (29%) were women. The mean age of the patient cohort was 67.5±8.6 years (median: 67 years, range: 39–88 years). Tumors were typed according to Lauren classification as intestinal in 30 (45%) and diffuse in 36 (55%) patients [27]. The mean age was 66.2±9.5 years (median: 60 years, range: 39–81 years) and 68.9±7.4 years (median: 73 years, range: 57–88 years) for patients with diffuse and intestinal type of gastric cancer, respectively. Three levels of differentiation were used to classify grading as: well, moderately and poorly differentiated. Tumors staging was assessed using the 5th edition of the Tumor, Node, Metastasis (TNM) system according to the Union Internationale Contra la Cancrum (UICC) and the American Joint Committee on Cancer (AJCC) [28]. Twenty-eight patients with intestinal and 35 with diffuse type gastric cancer were followed up, with the length of the follow up varying from 1 to 104 months (mean 38.7±24.9 median 53 months) and 1 to 56 (mean 19.4±15.9 median 28 months), respectively. All the examined clinicopathological characteristics are reported in Tables 1 and 2.

Immunohistochemistry

Immunostainings for FAK were performed on paraffin-embedded tissue sections using an appropriate mouse anti-human FAK antibody, raised against the COOH-terminal of

Table 1 Associations of FAK expression with clinicopathological characteristics in intestinal type gastric adenocarcinoma cases

Clinicopathological parameters	FAK expression		
(n=30)	Low (%)	High (%)	p-value
Patients	13 (43)	17 (57)	
Age			0.961
<68.9	7 (23)	9 (30)	
≥68.9	6 (20)	8 (27)	
Gender			0.222
Men	11 (37)	11 (37)	
Women	2 (6)	6 (20)	
Histological grade			0.197
well + moderately differentiated	9 (30)	15 (50)	
poorly differentiated	4 (13)	2 (7)	
pT classification			0.225
T1-2	9 (30)	8 (27)	
T3-4	4 (13)	9 (30)	
pN classification			0.245
N0-1	12 (40)	17 (57)	
N2	1 (3)	0 (3)	
pM classification			0.374
M0	13 (43)	16 (54)	
M1	0 (0)	1 (3)	
pStage			0.346
I	6 (20)	5 (17)	
II–IV	7 (23)	12 (40)	
Ki-67 protein statement			0.012
Ki-67 below mean (<57%)	9 (30)	4 (13)	
Ki-67 over mean (≥57%)	4 (13)	13 (44)	

FAK protein (sc-1688, Santa Cruz Biotechnology, Santa Cruz, CA, USA), a Vectastain Elite ABC-peroxidase kit (Vector Laboratories, Peterborough, United Kingdom) and the Liquid DAB Substrate-Chromogen System (DAKO, Glostrup, Denmark) according to manufacturers' instructions. As positive and negative controls, known positive and negative cases of our previously study were applied [13]. The tumors proliferative capacity was assessed immunohistochemically, using a mouse anti-human Ki-67 antigen IgG_{1k} antibody (clone MIB-1, Dakopatts, Glostrup, Denmark) and the same procedure used for FAK protein detection [29]. Antigen retrieval (citrate buffer at pH 6.1 and microwave heating) was performed before incubation with both primary antibodies anti-FAK and anti-Ki-67. The sections were counterstained with Harris hematoxylin (Merck, Darmstadt, Germany). Additionally, the specificity of FAK staining was verified by the use of an isotype specific secondary antibody (anti-mouse IgG_1).

Evaluation of Immunohistochemistry

Stained sections were independently assessed by S.T. and S.V. blinded to the clinical data with complete observers' agreement. The percentage of positively stained cells in immunohistochemistry experiments were obtained by counting at least 1,000 cells in each case. Specimens were considered "positive" for FAK protein when more than 5% of tumoral cells were stained. Gastric adenocarcinoma cases were stratified into two groups according to the amount of FAK positive tumor cells: a group of low FAK expression when less than the mean percentage value of tumoral cells was positively stained and a group of high FAK expression when more than the mean percentage value of tumoral cells was positively stained. In FAK-positive cases, the intensity of staining was also estimated and graded in a three step scale as mild (+), moderate (++) and intense (+++).

Statistical Analysis

Chi-square tests were used to assess the association of FAK expression (low *vs* high) and staining intensity (mild *vs* moderate or intense) with clinicopathological variables. Survival curves were constructed using the Kaplan-Meier method and compared using the log-rank test. Cox

Table 2 Associations of FAK staining intensity with clinicopathological characteristics in FAK-positive intestinal type gastric adenocarcinoma cases

Clinicopathological parameters	FAK intensity		
(n=25)	Mild (%)	Moderate & Intense (%)	p-value
Patients	12 (48)	13 (52)	
Age			0.848
<68.9	6 (24)	7 (28)	
≥68.9	6 (24)	6 (24)	
Gender			0.471
Men	9 (36)	8 (32)	
Women	3 (12)	5 (20)	
Histological grade			0.238
well + moderately differentiated	9 (36)	12 (48)	
poorly differentiated	3 (12)	1 (4)	
pT classification			0.821
T1-2	7 (28)	7 (28)	
T3-4	5 (20)	6 (24)	
pN classification			[a]
N0-1	12 (48)	13 (52)	
N2	0 (0)	0 (0)	
pM classification			0.327
M0	12 (48)	12 (48)	
M1	0 (0)	1 (4)	
pStage			0.471
I	3 (12)	5 (20)	
II–IV	9 (36)	8 (32)	
Ki-67 protein statement			0.327
Ki-67 below mean (<57%)	6 (24)	4 (16)	
Ki-67 over mean (≥57%)	6 (24)	9 (36)	

[a] No enough data to perform statistical analysis

proportional hazard regression analysis was used to evaluate the effect of FAK expression and staining intensity as prognostic factors on patients' survival. A two-tailed $P<0.05$ was considered (statistically) significant. Statistical analyses were performed using the software package SPSS for Windows (version 11.0; SPSS Inc., Chicago, IL, USA).

Results

Concerning the whole number of gastric adenocarcinoma cases, which includes both histological types, FAK positivity was noted in 57 out of 66 (86%) specimens. The mean FAK expression value was 44%, while the incidence of tumors with high FAK expression was 48% (32 out of 66 cases). FAK staining presented mainly cytoplasmic and occasionally membraneous pattern. Representative tumor cells stained for FAK protein in intestinal and diffuse type of gastric cancer cases are depicted in Fig. 1a and b, respectively. In FAK-positive cases, in which both histological types are included, the intensity of immunostaining was further classified as mild in 27 (47%), moderate in 20 (35%) and intense in 10 (18%) out of 66 cases.

Among the different histological types, 25 (78%) out of 30 intestinal type and 32 (89%) out of 36 diffuse type adenocarcinoma cases were FAK-positive. High FAK expression was more frequently detected in intestinal (57%) than in diffuse (42%) type adenocarcinomas; however, this difference was not significant ($P>0.05$). Cases of diffuse type carcinoma presented more frequently mild or moderate (91%) FAK staining intensity compared to intestinal (72%) type without though reaching statistical significance ($P>0.05$). In fact, among the 25 FAK-positive intestinal type carcinoma cases, 12 (48%) presented mild, 6 (24%) moderate and 7 (28%) intense staining intensity. Among the 32 FAK-positive diffuse type carcinoma cases, 15 (47%) presented mild, 14 (44%) moderate and 3 (9%) intense staining intensity. All gastric adenocarcinoma cases were Ki-67 positive, presenting nuclear pattern of staining. The mean Ki-67 value was 53% and 57% in diffuse and intestinal type carcinoma cases, respectively.

In intestinal type adenocarcinomas, we did not find any significant association between the extent of FAK expression (low *vs* high) and patients' age and gender, tumor histopathological grade, disease stage, tumor size, nodal status and the presence of organ metastasis (Table 1). FAK expression was significantly associated with tumor proliferative capacity assessed by Ki-67 labeling index, as high FAK-expressing tumors more frequently presented increased proliferative capacity ($P=0.012$) (Table 1). No significant associations between FAK staining intensity (mild *vs* moderate or intense) and the clinicopathological characteristics examined were noted (Table 2).

In diffuse type adenocarcinomas, we did not find any significant association between the extent of FAK expression and patients' age, tumor histopathological grade, disease stage, tumor size, nodal status, the presence of organ metastasis and tumor proliferative capacity (Table 3). There was only a borderline association between FAK expression and patients' gender ($P=0.058$), as men more frequently presented high FAK expression levels compared to women (Table 3). In FAK-positive diffuse type adenocarcinomas, FAK staining intensity was statistically significantly associated with tumor size ($P=0.026$) and disease stage ($P=0.024$), presenting also borderline associations with patients' gender ($P=0.077$) and nodal status ($P=0.053$) (Table 4). In fact, mild FAK staining intensity was more frequently detected in adenocarcinoma cases with larger tumor size and advanced disease stage, as well as the presence of lymph node metastases. Moderate or intense FAK staining intensity was more frequently detected in men compared to women. FAK staining intensity was not associated with the remaining clinicopathological characteristics examined (Table 4).

Concerning the whole number of gastric adenocarcinoma cases, in which both histological types are included ($n=66$), the Kaplan-Meier product-limit method for overall analysis survival according to FAK expression (low *vs* high) and intensity of immunostaining (mild *vs* moderate and intense) did not reveal statistically significant correlations (log-rank test, $P=0.718$ and $P=0.741$, respectively) (data not shown).

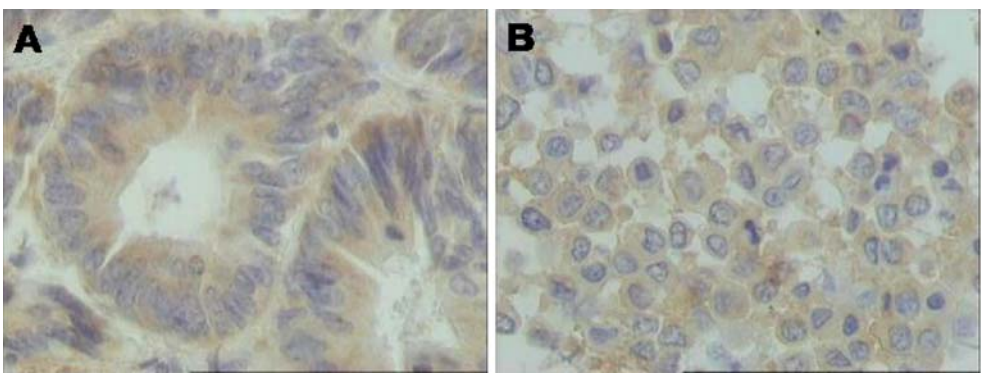

Fig. 1 Representative cases of cytoplasmic FAK protein expression in tumor cells of gastric adenocarcinoma: **a** Intestinal type. **b** Diffuse type. Streptavidin-biotin-peroxidase, DAB chromogen, Harris hematoxylin counterstain (original magnification X400)

Table 3 Associations of FAK expression with clinicopathological characteristics in diffuse type gastric adenocarcinoma cases

Clinicopathological parameters	FAK expression		
(n=36)	Low (%)	High (%)	p-value
Patients	21 (58)	15 (42)	
Age			0.770
<66.2	8 (22)	5 (14)	
≥66.2	13 (36)	10 (28)	
Gender			0.058
Men	12 (33)	13 (36)	
Women	9 (25)	2 (6)	
Histological grade			0.558
well + moderately differentiated	6 (17)	3 (8)	
poorly differentiated	15 (43)	12 (34)	
pT classification			0.418
T1-2	7 (19)	7 (19)	
T3-4	14 (30)	8 (23)	
pN classification			0.359
N0-1	20 (55)	13 (36)	
N2	1 (3)	2 (6)	
pM classification			0.650
M0	18 (50)	12 (34)	
M1	3 (8)	3 (8)	
pStage			0.589
I	4 (11)	4 (11)	
II–IV	17 (47)	11 (31)	
Ki-67 protein statement			0.418
Ki-67 below mean (<53%)	14 (39)	8 (23)	
Ki-67 over mean (≥53%)	7 (19)	7 (19)	

Stratifying for each histological type separately, in diffuse type gastric cancer cases, high levels of FAK expression were significantly associated with longer overall survival times (log-rank test, P=0.014) (Fig. 2a) and proved to be a significant indicator of favourable prognosis in multivariate analysis (Cox regression analysis, P=0.016). In contrast, in intestinal type gastric cancer cases, high levels of FAK expression showed a trend to be correlated with shorter overall survival times (log-rank test, P=0.092) (data not shown). No significant associations of FAK staining intensity and patients' survival were noted in either intestinal or diffuse type of gastric adenocarcinoma patients (data not shown).

Discussion

It is certainly well-established that FAK is overexpressed in various tumors, while unopposed FAK signalling promotes tumor growth, progression, metastasis and angiogenesis. There is also substantial evidence to support that FAK expression is upregulated during transformation of normal tissue to malignant state [8, 12, 23, 30–32]. In normal cells, FAK activity is considered to be under constant regulation by mechanisms such as gene amplification, alternative splicing and action of phosphatases; however, in transformed cells unopposed FAK signalling promoted cancer cells' malignant characteristics which promote tumor growth, progression and metastasis [8, 9]. Currently, several *in vivo* studies have evaluated the diagnostic and prognostic significance of FAK expression in a variety of cancer types, underlying the crucial role of FAK in cancer biology [8].

The present study revealed high immunoreactivity of FAK protein in gastric cancer specimens, in which both histological types are included, as 86% of the examined cases were FAK positive, presenting moderate or intense intensity of immunostaining in more than a half (53%) of them. Moreover, a high incidence (48%) of tumors expressing high FAK protein levels was noted. Thus, it is speculated that FAK could have potential roles in the progression of gastric cancer. The current incidence of gastric adenocarcinoma FAK positivity is among the highest incidences already been reported for all types of carcinomas, while a similar incidence for the intensity of staining was found for this specific type of tumor

Table 4 Associations of FAK staining intensity with clinicopathological characteristics in FAK-positive diffuse type gastric adenocarcinoma cases

Clinicopathological parameters	FAK staining intensity		
(n=32)	Mild (%)	Moderate & Intense (%)	p-value
Patients	15 (47)	17 (53)	
Age			0.755
<66.2	7 (22)	7 (22)	
≥66.2	8 (25)	10 (31)	
Gender			0.077
Men	8 (25)	14 (44)	
Women	7 (22)	3 (9)	
Histological grade			0.838
well + moderately differentiated	4 (13)	4 (13)	
Poorly differentiated	11 (34)	13 (40)	
pT classification			0.026
T1-2	3 (9)	10 (31)	
T3-4	12 (38)	7 (22)	
pN classification			0.053
N0-1	12 (38)	17 (53)	
N2	3 (9)	0 (0)	
pM classification			0.190
M0	14 (44)	13 (41)	
M1	1 (3)	4 (12)	
pStage			0.024
I	1 (3)	7 (22)	
II–IV	14 (44)	10 (31)	
Ki-67 protein statement			0.688
Ki-67 below mean (<53%)	9 (28)	9 (28)	
Ki-67 over mean (≥53%)	6 (19)	8 (25)	

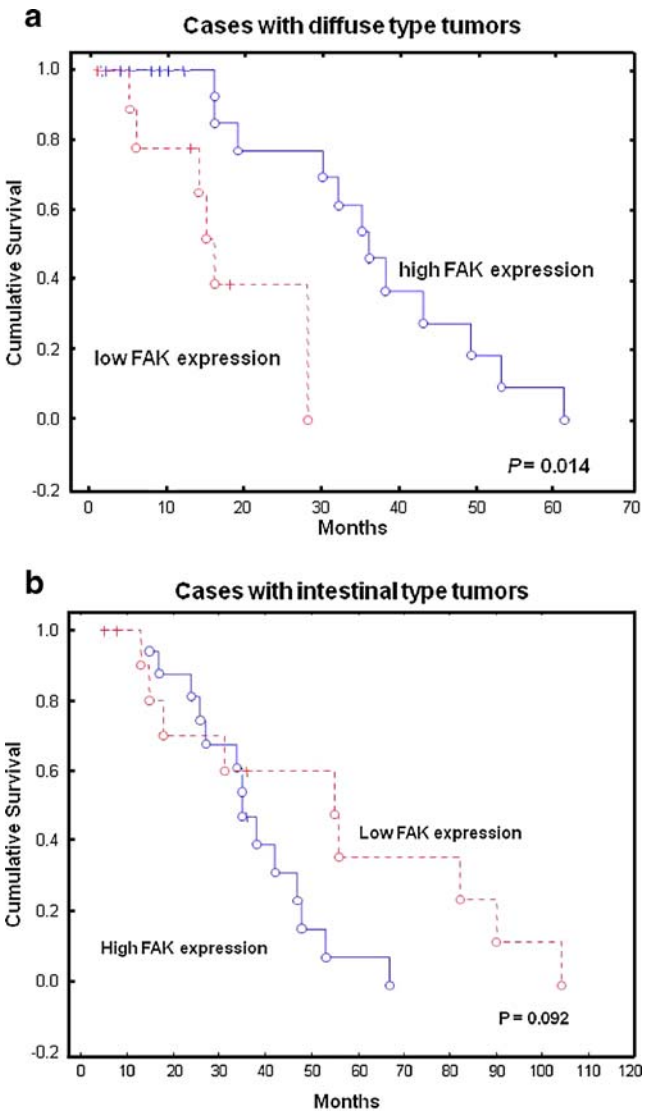

Fig. 2 Kaplan-Meier survival analysis stratified according to FAK expression in patients with gastric cancer. **a** Subgroup of diffuse type cases. **b** Subgroup of intestinal type cases. Low FAK expression is depicted by red dashed line, while high FAK expression is depicted by blue continuous line

malignancy [23]. Beside this, Su et al. conducted an immunohistochemical study on 75 gastric cancer cases, revealing for the first time significant associations between enhanced FAK expression and poor differentiation, deep invasion, and lymph node metastasis. However, none other information about the prognostic significance of FAK immunoreactivity was provided [23]. Moreover, the aforementioned study did not distinguish the examined gastric carcinoma cases according to Lauren classification in order to analyze the clinical relevance of FAK immunoreactivity in intestinal and diffuse type carcinomas separately. In this context, we documented that high FAK expression was more frequently detected in intestinal than in diffuse adenocarcinomas. Moreover, diffuse type cases more frequently presented mild or moderated FAK staining intensity than intestinal ones. However, these differences were not statistically significant in order to obtain a clear discrimination between intestinal and diffuse type carcinomas according to FAK expression or staining intensity.

In intestinal type gastric adenocarcinoma cases, we showed that enhanced FAK expression was significantly associated with increased tumor proliferative capacity. This result is in line with previous evidence where FAK-overexpressing tumor cells presented increased proliferating capacity compared to non-overexpressing ones in several types of cancer, such as esophageal and breast cancer [31, 32]. There is also substantial evidence which supports that FAK may contribute to uninhibited proliferation of cancer cells mainly through the Extracellular-regulated kinase (Erk) signaling pathway [8, 9]. Indeed, in human glioblastoma cells, FAK overexpression, *in vivo*, promoted Erk activity and increased the transcription of the Kuppel—like factor 8 (KLF8), which directly activated cyclin-D1 transcription and thus promoted cell proliferation [33]. Beside this, the FAK dominant negative, FAK-related non-kinase (FRNK), expression suppressed the growth of human tumor cells in nude mice [34].

In diffuse gastric adenocarcinoma cases, mild FAK staining intensity was more frequently detected in cases with larger tumor size, more than one lymph node metastases and advanced disease stage. Moreover, men were more frequently characterized by high FAK expression, as well as moderate or intense FAK staining intensity compared to women. The significant association between FAK staining intensity and tumor size supports evidence that FAK could be related with the tumor burden, but not with the tumor biological behavior in this histological type of gastric cancer. Accordingly, previous evidence revealed significant association of FAK immunoreactivity with tumor size in several types of neoplasia. More to the point, FAK overexpression, assessed by immunohistochemistry, was significantly correlated with large tumor size in liver and pancreatic cancer, as well as in intrahepatic cholangiocarcinoma [14, 15, 35]. FAK mRNA levels, assessed by Western blot, were also significantly associated with tumor size in non-small cell lung cancer patients [36]. It was also reported that astrocytoma cells expressing FAK formed larger tumors in nude mice than in tumor cells derived from the parental cell lines [37], while expression of a hyperactive mutant of FAK in a breast cancer cell line resulted in elevated tumor size in nude mice [38]. There is also evidence that FAK immunoreactivity was associated with disease stage and lymph node metastasis in several types of neoplasia, such as esophageal, breast, lung, ovarian and colon carcinoma [31, 32, 35, 39, 40].

As regards the prognostic value of FAK immunoreactivity, controversial results have been reported among the different

types of cancer. In this context, weak FAK expression was associated with worse prognosis in invasive cervical cancer and intrahepatic cholangiocarcinoma patients [17, 35]. In contrast, higher FAK expression levels were significantly associated with shorter survival times in liver and ovarian carcinoma patients [15, 39]. On the other hand, FAK expression did not predict patients' outcome in node negative breast cancer [41] or in resectable pancreatic cancer patients [14]. Furthermore, although elevated FAK expression levels presented poorer survival rates in esophageal and head and neck squamous cell carcinoma patients, this relationship did not reach statistical significance [31, 42].

In the current study, the survival analysis of FAK protein expression and intensity of immunostaining concerning the whole number of gastric adenocarcinoma cases, in which both histological types are included, did not reveal significant associations. However, in diffuse type, patients with tumors expressing high FAK levels were characterized by significantly longer overall survival times. On the other hand, in intestinal type, patients with tumors expressing high FAK levels were characterized by shorter overall survival times without though reaching statistical significance. The contradictory prognostic value of FAK expression between diffuse and intestinal gastric cancer types could be ascribed to the different signals produced from the tumor microenvironment and the individual cellular characteristics of each tumor type. Such distinct characteristics could trigger tumoral cells to upregulate or downregulate FAK signalling in respect to tumor histological type. Current substantial evidence also suggested that multiple genetic and epigenetic alterations in oncogenes, tumor-suppressor genes, cell-cycle regulators, cell adhesion molecules, DNA repair genes and genetic instability, are differentiated according to the histological type of gastric cancer, indicating that intestinal and diffuse carcinomas exhibit dissimilar carcinogenetic pathways and may be considered as distinct clinical and epidemiological entities [24–27]. In general, patients with diffuse type gastric cancer were characterized by shorter survival times compared with those diagnosed with intestinal one [43, 44]. In this context, we found a borderline association between tumor histological type and patients' survival (log-rank test, P=0.083), which further supports the distinct prognostic impact of the tumor histological type on the current clinical material.

As far as concerned the use of FAK signaling as target for anticancer therapy, in breast cancer cells transduction of adenovirus containing COOH-terminal domain of FAK (FAK-CD) resulted in loss of adhesion, degradation of FAK, and induction of apoptosis in FAK overexpressing cells [45]. From a pharmacologic point of view, substantial evidence was demonstrated that a constructed P^{125FAK} ribozyme decreased FAK gene expression and induced apoptosis in human gastric cancer cells, *in vitro* [46]. In this context, it was shown that ribozyme, a small RNA molecule presenting catalytic activity, can be combined with complementary sequences of mRNA to block the translation of mRNA, while it can also incise mRNA and promote its degradation [47]. Drugs used in cancer chemotherapy, besides their basic mode of action, were also shown to act through altering FAK signaling [9]. New perspectives have also been unfolded by the transfection of cancer cells' with fak mutants or genes that suppress FAK expression or activity, such as PTEN, RRM1 and mda-7 [9]. Overall, these data support substantial evidence for possible use of FAK targeting in anticancer therapy.

In conclusion, the present study revealed high immunoreactivity of FAK protein in gastric neoplasia, supporting also evidence for a potential role of FAK protein in the diagnosis and prognosis of gastric cancer patients. The clinical impact of FAK immunoreactivity was found different between the two distinct histological entities of gastric cancer, which further reflected their individual characteristics. However, the current data should be confirmed by a larger cohort study conducted on each histological type separately in the aim to evaluate whether FAK expression could be considered as a tumor marker in clinical settings. Further research effort examining also the phosphorylation status of FAK protein should be warranted to delineate whether FAK could constitute a potent target for future therapeutic approaches in gastric neoplasia.

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