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RESEARCH

Expression and Clinical Significance of FAK and Src Proteins in Human Endometrial Adenocarcinoma

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Abstract Focal Adhesion Kinase (FAK) is a protein tyrosine kinase, localised in the focal adhesions, which, upon activation interacts with Src, another tyrosine kinase, regulating several cellular signalling pathways. Both enzymes have been implicated in malignant transformation and disease progression. The aim of the present study was to evaluate the clinical significance of FAK and Src expression in cases of endometrial adenocarcinoma. The total (t) and the activated, phosphorylated (p) forms of FAK and Src proteins were assessed immunohistochemically in tumour specimens obtained from 43 endometrial adenocarcinoma patients and were statistically analyzed in relation to various clinicopathological parameters and tumour proliferative capacity, reflected by Ki-67 labelling index. t-FAK positivity was significantly correlated with FIGO disease stage (p=0.031), and t-FAK overexpression with patients' age (p=0.015). No statistically significant correlation was identified between t-FAK staining intensity, t-Src positivity, overexpression or staining intensity and any of the clinicopathological parameters tested. No significant correlation was found between neither the positivity nor the intensity of staining of either p-FAk or p-Src with any of the parameters under study. Nonetheless, important, but non-significant, trends were identified between t-FAK staining intensity, t-Src positivity and overexpression and patients' survival (log rank, p=0.122, p=0.090 and p=0.057 respectively). Similarly, p-FAK and p-Src staining characteristics seemed to correlate, even though non-significantly, with patients' survival (log rank, p=0.051 and p=0.070 for p-FAK and p-Src expression, respectively; log rank, p=0.134 and p=0.110 for p-FAK and p-Src staining intensity, respectively). These results support an important potential role of FAK-Src signalling in endometrial malignant disease progress and render further research in this field a necessity.

Keywords FAK · Src · Clinical significance · Immunohistochemistry · Endometrial adenocarcinoma

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Introduction

Focal adhesion kinase (FAK) was first described in 1992 [1, 2] as a member of the protein tyrosine kinases (PTKs) family and particularly of the nonreceptor PTKs subfamily [3–5]. The cDNA of FAK, mapped on human chromosome 8, encodes a protein with a predicted molecular weight of 119-121 kDa depending on species, though on the basis of its migration in gels it is known as $p125^{FAK}$ [6]. FAK is expressed in a variety of species, tissues and cell types [6–8]. A feature of FAK is its subcellular localisation to specialized submembranous structures called focal adhesions (FAs) [6]. Unlike many other PTKs, FAK does not have SH2 or SH3 domains, but it does have SH2 and SH3 domain-interacting phosphotyrosines and proline-rich regions, respectively [9], by which, when activated,

interacts with other proteins, with Src being the most important.

Src is also a non-receptor PTK, encoded by the gene *c-Src*. Src SH2 domain is available for binding with phosphotyrosine residues of other molecules such as c-Met and FAK [10, 11]. The PTK v-Src is the transforming product of Rous sarcoma virus, the first identified oncogenic retrovirus. v-Src differs from Src by substitution of sequences at the C-terminus, which leads in the loss of the amino acids that normally bind to the SH domains and stabilize the inactive conformation of the molecule, resulting in the constitutively enzymatic activation [12, 13]. Along with FAK, Src has been shown to regulate many complex cell signalling pathways.

FAK was shown to be activated by integrins, as well as by other cellular stimuli, substances, receptors and under various pathological conditions, able to generate signals through either G-protein linked receptors, transmembrane growth factor receptors or through unknown mechanisms [14]. Integrin clustering results in conformational changes in the molecule of FAK leading to autophosphorylation of tyrosine residue 397 and the subsequent recruitment of Src, resulting in the phosphorylation of FAK at several tyrosine residues and full catalytic FAK activation [14]. The FAK/Src complex binds and phosphorylates many downstream molecules, such as $p130^{Cas}$, Grb2 and PI3K, thereby transducing signals down many different, complex pathways, thus regulating various basic cellular functions, such as cell proliferation and growth, protection from apoptosis, adhesion, spreading, invasion and migration [6, 7, 9, 14, 15]. Furthermore, FAK signaling has been shown to mediate trophoblast development [16], embryogenesis and morphogenesis [17–19].

Both Src and FAK seem to play a crucial role in the malignant transformation and disease progression. Increased Src expression has been reported in several human tumours including colorectal [20], pancreatic [21] and breast [22] cancer, while increased Src activity has been implicated in the promotion of the malignant phenotypic characteristics of different cancer cell types [23–25]. Similarly, many studies suggested the role of FAK in various human malignancies [26]. Accumulating data from *in vitro* and *in vivo* studies render FAK and Src as possible targets for anticancer therapy [27, 28].

More specifically, for endometrial neoplasia, evidence were presented that FAK played a role in endometrial carcinogenesis, while FAK overexpression correlated with two independent prognostic factors for endometrial carcinoma, p53 and histological grade [29]. On the other hand, another study failed to show any statistical significant association between FAK staining intensity and the uterocervical tumour grade of differentiation or the depth of invasion [30]. However, no studies have yet questioned the expression or the clinical significance of Src or of the activated, phosphorylated forms p-FAK and p-Src in this type of neoplasia.

In the present study, immunohistochemical methods were used to assess the expression of the total (t) and phosphorylated (p) FAK and Src proteins on tumour samples obtained from patients with endometrial carcinoma. Positivity, overexpression and staining intensity for t-FAK, t-Src and their phosphorylated forms (p-FAK and p-Src) were associated with various clinicopathological parameters and the tumour proliferative capacity, assessed by Ki-67 labelling index, in the aim to delineate their clinical significance in endometrial neoplasia.

Patients and Methods

Clinical Material

Forty-three patients with endometrial carcinoma constituted the group of our study, with a mean age of 63.8±9.38 years (range 82–40). Thirty-four patients had adenocarcinoma (79.1%) and 9 adenocarcinoma with squamous elements (20.9%). The cases were classified as well, 12 (27.9%); moderately, 24 (55.8%); and poorly differentiated, 7 (16.3%). According to the IUCC classification FIGO stage 5 cases were characterised as stage 1a (11.6%), 14 stage 1b (31.8%), 15 stage 1c (34.1%), 3 stage 2a (6.8%), 2 stage 2b (4.5%), 2 stage 3a (4.5) and 2 stage 4a (4.5%). Based on morphological feature and intense p53 immunoreactivity, 31 (72.1%) patients presented type I endometrioid endometrial cancer, while the remaining 12 (27.9%) patients had type II seropapillary form. No patient received any anticancer treatment prior to the operation. The patients were followed up for a time interval of 22 to 72 months (mean: 63.7±14.8 months).

Immunohistochemistry

Formalin-fixed, paraffin-embedded 4-μm thick tissue sections were dewaxed in xylene and brought to water through graded alcohols. The endogenous peroxidase activity was removed by treating the sections with freshly prepared 0.3% hydrogen peroxide in methanol in the dark for 30 min, at room temperature. Non-specific antibody binding was then blocked using a specific blocking reagent for 5 min (Snipper; Biocare Medical, Walnut Creek, CA). A mouse anti-human t-FAK IgG_1 antibody, raised against the COOH-terminal of FAK protein (sc-1688, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and another mouse anti-human c-Src IgG_{2a} antibody (sc-5266, Santa Cruz Biotechnology) were used to assess the total forms of FAK (t-FAK) and Src (t-Src) proteins, respectively. A rabbit polyclonal anti-phospho FAK (Tyr 861,

Stressgen Corporation Bioreagents, Glanford Ave, Victoria, Canada) and another rabbit polyclonal anti-phospho Src (Tyr 418, Stressgen Corporation Bioreagents) were used to assess the phosphorylated forms of FAK (p-FAK) and Src (p-Src) proteins, respectively. A mouse anti-human Ki-67 antigen IgG_{1k} antibody (clone MIB-1, Dakopatts, Glostrup, Denmark) was also used to assess tumor proliferative capacity. Antigen retrieval was performed for all primary antibodies by microwaving slides in 10 mM citrate buffer (pH 6.0) for 15 min at 720 W according to manufacturer's instructions [31].

The sections were then incubated for 1 h at room temperature with the primary antibodies, diluted 1:100 (t-FAK, p-FAK) and 1:300 (t-Src, p-Src) in phosphate buffered saline (PBS). After washing three times with PBS, the sections were incubated at room temperature with biotinylated linking reagent (Biocare Medical) for 10 min, followed by incubation with peroxidase conjugated streptavidin label (Biocare Medical) for 10 min. The resultant immune peroxidase activity was developed in 0.5% 3,30-diaminobenzidine hydrochloride (DAB; Sigma, Saint Louis, MO) in PBS containing 0.03% hydrogen peroxide for 3 min. The sections were then counterstained with Harris' hematoxylin and mounted in Entellan (Merck, Darmstadt, Germany). Appropriate negative controls were performed by omitting the primary antibody and/or substituting it with an irrelevant anti-serum. Pancreatic cancer tumour specimens with enhanced FAK and Src expression were used as positive controls [32].

Evaluation of Immunohistochemistry

Stained sections were independently assessed by ST and IS blinded to the clinical data with complete observers' agreement. The cells were counted at high power magnification (X200). Specimens were considered positive for t-FAK, t-Src, p-FAK, p-Src and Ki-67 proteins when more than 5% of the tumour cells within the section were positively stained [31, 32]. The specimens were characterised to present "overexpression" for all the examined proteins when the expression percentage value was higher than the median value of percentage expression in all cases (40% for t-FAK, 30% for Ki-67 and 15% for t-Src) [31, 32]. The levels of expression of both phosphorylated forms in all of the positive specimens were generally low, consequently no overexpression was identified. The intensity of t-FAK, t-Src, p-FAK and p-Src staining was graded as weak (+), moderate (++), or strong (+++) [31, 32].

Statistical Analysis

Significant associations between FAK and Src staining characteristics and the clinicopathological parameters and tumour proliferative capacity were investigated with the use of the appropriate statistical test among Pearson's chi-square, Kruskal-Wallis H-test and Spearman's correlation. The exact method was consistently used for all the statistical tests. The Kaplan-Meier method was used to calculate the survival curves between patients with tumours stained positive and negative for the two enzymes and their phosphorylated forms; with tumours overexpressing the enzymes and not; and between those with high (moderate and strong) and low (zero and weak) intensity of staining. A 2-tailed $P<0.05$ was considered (statistically) significant. Statistical analyses were performed using the software package SPSS for Windows (version 13.0; SPSS Inc., Chicago, IL, USA).

Results

t-FAK staining was found positive in 35 out of 43 (81.4%) cases, while t-Src staining was positive in 21 (48.8%) cases (Table 1). Representative immunostainings for t-FAK and t-Src proteins' expression in endometrial adenocarcinoma are depicted in Fig. 1. Endometrial cancer cells exhibited mainly cytoplasmic and occasionally membranous immunoreactivity for both t-FAK and t-Src enzymes. t-FAK overexpression was noted in 22 (51.2%) and t-Src in 19 (44.2%) cases (Table 2). Eight-teen (41.9%) specimens exhibited weak t-FAK immunoreactivity and 17 (39.5%) moderate (Table 3). With regards to t-Src staining intensity, 14 (32.6%) endometrial carcinoma specimens presented weak, 7 (16.3%) moderate intensity of immunostaining (Table 4). After statistical analysis, t-FAK positivity correlated significantly only with the FIGO stage of the disease ($p=0.031$) (Table 1). A significant correlation was also identified between t-FAK overexpression and patients' age ($p=0.015$) (Table 2). On the other hand, statistical analysis failed to show any significant correlations between t-Src positivity, overexpression and intensity and any of the clinicopathological characteristics examined (Tables 1, 2 and 4). A detailed description of the results can be found on Tables 1, 2, 3 and 4.

When the specimens were stained for the activated-phosphorylated forms of the two enzymes, 7 (16.3%) expressed p-FAK and 11 (25.6%) expressed p-Src (Table 5). Representative immunostainings for p-FAK and p-Src proteins' expression in endometrial adenocarcinoma are depicted in Fig. 2. Endometrial cancer cells exhibited mainly cytoplasmic and occasionally membranous immunoreactivity for both p-FAK and p-Src enzymes. With regards to the staining intensity, 6 (14.0%) exhibited weak and 1 (2.3%) moderate immunoreactivity for p-FAK, while 3 (7%) and 8 (18.6%) for p-Src respectively. Statistical analysis failed to identify any significant correlation

Table 1 Correlations between t-FAK and t-Src positivity and clinicopathological characteristics in endometrial carcinoma cases

Clinicopathological characteristics	t-FAK positivity		*P* value	t-Src positivity		*P* value
	Positive (%)	Negative (%)		Positive (%)	Negative (%)	
Patients	35 (81.4)	8 (18.6)		21 (48.8)	22 (51.2)	
Age (mean), years			1.000			0.131
<64	17 (39.5)	4 (9.3)		13 (30.2)	8 (18.6)	
≥ 64	18 (41.9)	4 (9.3)		8 (18.6)	14 (32.6)	
Histological type			1.000			0.281
adenocarcinoma	27 (62.8)	7 (16.3)		15 (34.9)	19 (44.2)	
adenocarinoma with squamous elements	8 (18.6)	1 (2.3)		6 (14.0)	3 (7.0)	
Differentiation			0.754			0.349
Well	10 (23.3)	2 (4.7)		6 (14.0)	6 (14.0)	
Moderate	20 (46.5)	4 (9.3)		14 (32.6)	10 (23.3)	
Poor	5 (11.6)	2 (4.7)		1 (2.3)	6 (14.0)	
FIGO stage			0.031			0.369
1	30 (69.8)	4 (9.3)		18 (41.9)	16 (37.2)	
2	3 (7.0)	2 (4.7)		2 (4.7)	3 (7.0)	
3	0 (0)	2 (4.7)		0 (0)	2 (4.7)	
4	2 (4.7)	0 (0)		1 (2.3)	1 (2.3)	
Ki-67 protein statement			0.419			0.755
Ki-67 below median (<30%)	11 (25.6)	4 (9.3)		8 (18.6)	7 (16.3)	
Ki-67 above median (≥30%)	24 (55.8)	4 (9.3)		13 (30.2)	15 (34.9)	

between either the positivity or the intensity of either of the phosphorylated forms of the kinases and any of the parameters tested (Data not shown). Nonetheless, a non-statistical significant trend between p-Src staining intensity and the tumour cell's proliferative capacity was revealed (p=0.055, data not shown).

Patients' survival was further investigated with respect to t-FAK, t-Src, p-FAK and p-Src staining characteristics. In terms of t-FAK positivity, overexpression and intensity of immunostaining, the survival rate was not statistically different between the two groups compared (log rank, p=0.277, p=0.999 and p=0.122, respectively), even though a trend was revealed between t-FAK staining intensity and survival (p=0.122). Similarly, no significant difference in the survival rates was noticed with regards to the t-Src staining intensity (log rank, p=0.941), while a trend was identified between t-Src positivity and survival (log rank, p=0.090). t-Src overexpression marginally failed to correlate with survival (log rank, p=0.057). When the survival rates were compared with respect to p-FAK and p-Src staining characteristics, the positivity of p-FAK marginally failed to correlate with patients' survival (log rank, p=0.051), while a trend was identified with p-FAK staining intensity (log rank, p=0.134). Similarly, both the positivity and the staining intensity of p-Src showed a trend to correlate with survival, even though both failed to reach statistical significance (log rank, p=0.070 and p=0.110, respectively).

No significant differences were obtained between type I endometrioid and type II seropapillary endometrial cancer cases with respect to patients' age, histopathological type,

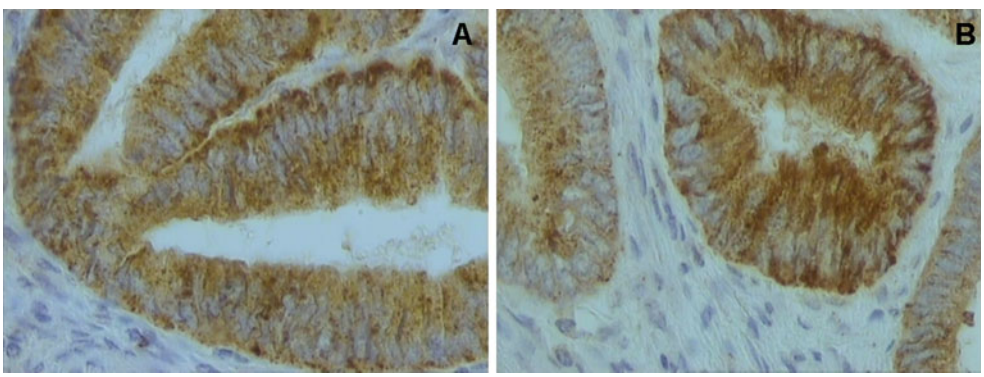

Fig. 1 Representative immunostainings for the total forms of **a** FAK and **b** Src expression in tumor cells of endometrial adenocarcinoma (original magnification X400). Endometrial cancer cells exhibited mainly cytoplasmic and occasionally membranous immunoreactivity for both enzymes

Table 2 Correlations between t-FAK and t-Src overexpression and clinicopathological characteristics in endometrial carcinoma cases

Clinicopathological characteristics	t-FAK overexpression		*P* value	t-Src overexpression		*P* value
	FAK overexpressed (level higher than the median; ≥40%)(%)	FAK not overexpressed (level lower than the median; <40%) (%)		Src overexpressed (level higher than the median; ≥15%)(%)	Src not overexpressed (level lower than the median; <15%) (%)	
Patients	22 (51.2)	21 (48.8)		19 (44.2)	24 (55.8)	
Age (mean), years			0.015			0.129
<64	15 (34.9)	6 (14.0)		12 (27.9)	9 (20.9)	
≥ 64	7 (16.3)	15 (34.9)		7 (16.3)	15 (34.9)	
Histological type			0.721			0.153
adenocarcinoma	18 (41.9)	16 (37.2)		13 (30.2)	21 (48.8)	
adenocarinoma with squamous elements	4 (9.3)	5 (11.6)		6 (14.0)	3 (7.0)	
Differentiation			0.153			0.246
Well	8 (18.6)	4 (9.3)		6 (14.0)	6 (14.0)	
Moderate	12 (27.9)	12 (27.9)		12 (27.9)	12 (27.9)	
Poor	2 (4.7)	5 (11.6)		1 (2.3)	6 (14.0)	
FIGO stage			0.242			0.491
1	19 (44.2)	15 (34.9)		16 (37.2)	18 (41.9)	
2	2 (4.7)	3 (7.0)		2 (4.7)	3 (7.0)	
3	0 (0)	2 (4.7)		0 (0)	2 (4.7)	
4	1 (2.3)	1 (2.3)		1 (2.3)	1 (2.3)	
Ki-67 protein statement			0.755			1.000
Ki-67 below median (<30%)	7 (16.3)	8 (18.6)		7 (16.3)	8 (18.6)	
Ki-67 above median (≥30%)	15 (34.9)	13 (30.2)		12 (27.9)	16 (37.2)	

grade and stage, as well as t-FAK, t-Src, p-FAK and p-Src staining characteristics (data not shown). Patients with type I endometrioid endometrial cancer showed slightly longer survival times compared to those with type II seropapillary form without reaching statistical significance (median survival times 71 months, IQR: 68–71 *vs* median survival times 68 months, IQR: 66–70). Tumor cell's proliferative capacity was significantly increased in seropapillary compared to endometrioid endometrial cancer cases (p=0.0026).

Finally, statistical analysis was separately performed in type I endometrioid and type II seropapillary endometrial cancer cases. In the subgroup of endometrioid cancer cases, t-FAK overexpression was significantly associated with patients' age and Ki-67 protein statement (p=0.0198 and p=0.0291, respectively). In the same subgroup, t-Src positivity and overexpression were significantly associated with histological type (p=0.0364 and p=0.0124, respectively), as adenocarcinoma cases with squamous elements showed enhanced t-Src immunostaining compared to adenocarcinoma cases without squamous elements. In the subgroup of seropapillary endometrial cancer cases, t-FAK positivity and staining intensity were significantly associated with FIGO stage (p=0.0074 and p=0.0206, respectively). The staining characteristics of the activated-phosphorylated forms of the two enzymes, p-FAK and p-Src, did not showed significant associations with the clinicopathological parameters examined (data not shown) except for an association between p-Src positivity and grade of differentiation (p= 0.0498). No associations between t-FAK, t-Src, p-FAK and p-Src staining characteristics and patients' survival were also noted (data not shown).

Discussion

The role of both kinases FAK and Src in human malignancy has been well established and current research projects are directed to the possible use of FAK- and Src-targeting molecules in anticancer therapy. Regarding the role of these molecules in endometrial carcinoma, data are still scarce. The study of Su et al. failed to show any statistical significant correlation between the t-FAK staining intensity and tumour differentiation or invasion [30]. On the other hand, Livasy et al. identified significant correlations between t-FAK overexpression and the two independent prognostic factors of endometrial carcinoma, the grade of differentiation and p53 overexpression [29]. Our study is even more analytic, as it investigates the possible associ-

Table 3 Correlations between t-FAK staining intensity and clinicopathological characteristics in endometrial carcinoma cases

Clinicopathological characteristics	t-FAK intensity			*P* value
	No (%)	Weak (%)	Moderate (%)	
Patients	8 (18.6)	18 (41.9)	17 (39.5)	
Age (mean), years				0.240
<64	4 (9.3)	7 (16.3)	10 (23.3)	
≥ 64	4 (9.3)	11 (25.6)	7 (16.3)	
Histological type				0.147
adenocarcinoma	7 (16.3)	13 (30.2)	14 (32.6)	
adenocarinoma with squamous elements	1 (2.3)	5 (11.6)	3 (7.0)	
Differentiation				0.386
Well	2 (4.7)	5 (11.6)	5 (11.6)	
Moderate	4 (9.3)	9 (20.9)	11 (25.6)	
Poor	2 (4.7)	4 (9.3)	1 (2.3)	
FIGO stage				0.170
1	4 (9.3)	16 (37.2)	14 (32.6)	
2	2 (4.7)	0 (0)	3 (7.0)	
3	2 (4.7)	0 (0)	0 (0)	
4	0 (0)	2 (4.7)	0 (0)	
Ki-67 protein statement				1.000
Ki-67 below median (<30%)	4 (9.3)	7 (16.3)	4 (9.3)	
Ki-67 above median (≥30%)	4 (9.3)	11 (25.6)	13 (30.2)	

ation between the positivity, overexpression and intensity of both total and activated FAK and Src and several clinicopathological parameters important for endometrial adenocarcinoma patients' management.

Most of the tumours, regardless their other characteristics under study, were found t-FAK positive, even if they were negative for t-Src staining. t-Src staining characteristics failed to correlate with any of the clinicopathological parameters tested. On the contrary, t-FAK expression correlated significantly with the FIGO disease stage, with low stage tumours expressing more frequently the kinase. This seemingly contradictory observation can be explained by focusing on the role of the enzyme in the progression of human malignancy, as it has been approached in research so far. FAK has been supported to promote the acquisition of a more aggressive behaviour by the tumour cells, regulating activities such as uninhibited proliferation, protection from apoptosis, epithelial to mesenchymal transition, invasion and metastasis. Therefore, the high expression rates noted in stage 1 tumours may signify that the cells in these tumours were in the process of acquiring all the necessary cellular characteristics necessary for the progress of the malignancy.

t-FAK overexpression was found significantly correlated with young patients' age, however this result may be directed by many confounding variables and it is unlikely to support any clinical significance. No significant correlation was revealed between t-FAK overexpression and any of the other parameters tested, including tumour grade.

Table 4 Correlations between t-Src staining intensity and clinicopathological characteristics in endometrial carcinoma cases

Clinicopathological characteristics	t-Src intensity			*P* value
	No (%)	Weak (%)	Moderate (%)	
Patients	22 (51.2)	14 (32.6)	7 (16.3)	
Age (mean), years				0.222
<64	8 (18.6)	8 (18.6)	5 (11.6)	
≥ 64	14 (32.6)	6 (14.0)	2 (4.7)	
Histological type				0.285
adenocarcinoma	19 (44.2)	11 (25.6)	4 (9.3)	
adenocarinoma with squamous elements	3 (7.0)	3 (7.0)	3 (7.0)	
Differentiation				0.280
Well	6 (14.0)	4 (9.3)	2 (4.7)	
Moderate	10 (23.3)	9 (20.9)	5 (11.6)	
Poor	6 (14.0)	1 (2.3)	0 (0)	
FIGO stage				0.195
1	16 (37.2)	11 (25.6)	7 (16.3)	
2	3 (7.0)	2 (4.7)	0 (0)	
3	2 (4.7)	0 (0)	0 (0)	
4	1 (2.3)	1 (2.3)	0 (0)	
Ki-67 protein statement				0.758
Ki-67 below median (<30%)	7 (16.3)	6 (14.0)	2 (4.7)	
Ki-67 above median (≥30%)	15 (34.9)	8 (18.6)	5 (11.6)	

Table 5 Correlations between p-FAK and p-Src positivity and clinicopathological characteristics in endometrial carcinoma cases

Clinicopathological characteristics	p-FAK positivity		*P* value	p-Src positivity		*P* value
	Positive (%)	Negative (%)		Positive (%)	Negative (%)	
Patients	7 (16.3)	36 (83.7)		11 (25.6)	32 (74.4)	
Age (mean), years			1.000			0.310
<64	3 (7.0)	18 (41.9)		7 (16.3)	14 (32.6)	
≥ 64	4 (9.3)	18 (41.9)		4 (9.3)	18 (41.9)	
Histological type			0.147			0.672
adenocarcinoma	4 (9.3)	30 (69.8)		8 (18.6)	26 (60.5)	
adenocarinoma with squamous elements	3 (7.0)	6 (14.0)		3 (7.0)	6 (14.0)	
Differentiation			0.219			0.799
Well	3 (7.0)	9 (20.9)		2 (4.7)	10 (23.3)	
Moderate	4 (9.3)	20 (46.5)		9 (20.9)	15 (34.9)	
Poor	0 (0)	7 (16.3)		0 (0)	7 (16.3)	
FIGO stage			0.270			0.311
1	7 (16.3)	27 (62.8)		10 (23.3)	24 (55.8)	
2	0 (0)	5 (11.6)		1 (2.3)	4 (9.3)	
3	0 (0)	2 (4.7)		0 (0)	2 (4.7)	
4	0 (0)	2 (4.7)		0 (0)	2 (4.7)	
Ki-67 protein statement			1.000			0.473
Ki-67 below median (<30%)	2 (4.7)	13 (30.2)		5 (11.6)	10 (23.3)	
Ki-67 above median (≥30%)	5 (11.6)	23 (53.5)		6 (14.0)	22 (51.2)	

Furthermore, no significant correlation was noted between t-FAK staining intensity and any of the clinicopathological parameters under study. The difference between these results and those previously published [29] could be explained in several ways. First of all, the different methods and materials used could account for such differences. Additionally, the lack of specimens from highly aggressive tumours, since patients with such neoplams rarely undergo surgical treatment, contributes to the same direction. Furthermore, a biological approach of the tumour's behaviour could explain these differences. Since FAK and Src regulate tumour cells' aggressiveness, less aggressive tumours need more the ample expression of such enzymes in order to overcome the barriers for their survival, invasion and metastasis. Additionally, most researchers agree that it is more likely that the activity of these enzymes is more important, rather than the extent or the amount of their expression. Specifically, for the endometrial carcinoma, our results failed to support a significant role for either of the enzymes for the disease progression, since no significant correlations were identified between the expression or the intensity of staining of the two kinases and any of the parameters under study. Nonetheless, an important observation was the trends (without reaching statistical significance) noticed between t-FAK and p-FAK staining intensity and patients' survival. Even more important was the marginally non-significant correlation between p-FAK positivity in tumour cells and the patients' survival rate. Similarly, t-Src positivity exhibited a trend to correlate with survival, as well as t-Src overexpression and p-Src positivity (even stronger, but yet not statistically significant association). Finally, a non-significant trend with patients'

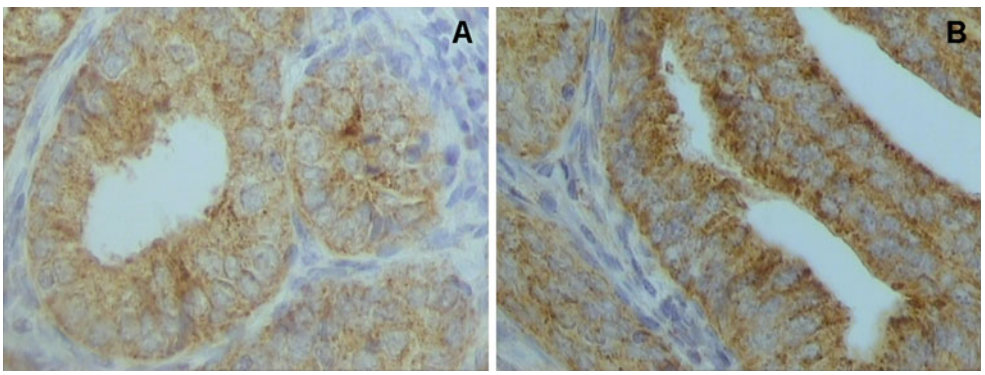

Fig. 2 Representative immunostainings for the phosphorylated forms of **a** FAK and **b** Src expression in tumor cells of endometrial adenocarcinoma (original magnification X400). Endometrial cancer cells exhibited cytoplasmic and occasionally membranous immunoreactivity for both enzymes

survival was identified also with p-Src staining intensity. These observations support the potential role of FAK-Src signalling pathways in the disease progression, including aspects that may affect patients' survival.

Moreover, it should be noted that endometrial cancers have biologically and histologically been classified into two main groups with different pathogenesis and prognosis [33]. Notably, several immunohistochemical and molecular markers have been reported to differentiate the two types [34]. In general, type I endometrioid endometrial cancer exhibits better prognosis compared to type II seropapillary form, while even the age of patients seems to be different between the two types [34]. Different genetic abnormalities, including alterations of TP53 genes, have further differentiated the two types and both types have been supported to be distinguished by the expression of several adhesion molecules, such as tight junction proteins and claudins [34]. In this aspect, the present study showed that t-FAK and t-Src, as well as p-FAK and p-Src staining characteristics were not capable of distinguishing the two endometrial cancer types. Nonetheless, analyzing separately the two endometrial cancer forms, we found indicative correlations between FAK and/or Src staining characteristics and certain clinicopathological parameters, such as patients' age, histological type, tumor cell's proliferative capacity (type I endometrioid form) and FIGO stage (type II seropapillary form). These findings highlight the emergent demand for larger cohort studies conducted on each type individually in order for precise conclusions to be drawn.

Conclusively, our study reveals a significant relationship between the expression of t-FAK and the stage of endometrial cancer. Of even more clinical significance are the data supporting the potential role of both FAK and Src in the pathophysiological aspects of the disease that affect patients' survival. The lack of detailed knowledge, along with the few data on this field, point out the necessity for further research in order to clarify the role of these two molecules and their potential use in the established therapeutic regimens of such malignancies.

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