

• GASTRIC CANCER •

Expression of focal adhesion kinase and $\alpha 5$ and $\beta 1$ integrins in carcinomas and its clinical significance

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Abstract

AIM: To detect the expression pattern of FAK (focal adhesion kinase) and integrin $\alpha 5$ and $\beta 1$ subunits in different kinds of cancerous tissues and to study their correlation with clinicopathological data including tumor type, grade and lymph node status. **METHODS:** Using an immunohistochemical technique, we examined the expression of FAK and integrin $\alpha 5$ and $\beta 1$ subunits in cancerous and noncancerous tissues obtained from 75 patients with gastric carcinomas, 21 colorectal carcinomas, 16 hepatocellular carcinomas, 20 uterocervical carcinomas, and 20 breast carcinomas.

RESULTS: The staining of FAK was stronger in cancerous than in noncancerous areas. Enhanced expression of FAK was detected in poor-differentiated carcinoma of the stomach and colorectum. Tumors with lymph node metastases had more FAK protein than those without metastases. In addition, the deeper the extent of tumor infiltration, the higher the FAK expression. The expression of integrin $\alpha 5$ and $\beta 1$ subunits was lower in cancerous areas than in noncancerous areas, but it was higher in well-differentiated cancerous tissues than in poor differentiated tissues. The relationship between the expression of integrin $\alpha 5$ and $\beta 1$ subunits and infiltration or metastasis was not significant. Cancerous tissues with stronger FAK expression (++ or +++) also had a higher expression of integrin $\alpha 5$ and $\beta 1$ subunits in the tumor and its unaffected margins.

CONCLUSION: FAK is a better marker for carcinogenesis and the progression of cancer than integrin $\alpha 5$ or $\beta 1$ subunit, and it may be not only a transformation-linked enzyme but also a progression-linked enzyme.

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INTRODUCTION

In recent years, much attention has focused on cell adhesion molecules. These studies have shown that adhesion molecules

affect cell behaviors and mediate signal transduction, especially in cancer cells. Integrins are a large family of cell surface receptors that are found in many animal species^[1]. They can affect signal transduction, cell proliferation, differentiation, survival and apoptosis, cell cycle and invasion and metastasis of carcinoma cells^[2-7]. Previous studies have provided a better understanding of the signaling pathways activated by integrins in adherent cells^[1]. As integrins bind to the extra cellular matrix (ECM), they become clustered in the plane of the cell membrane and activate a variety of non-receptor protein tyrosine kinases (PTKs), such as FAK, Abl, Syk and Src-family PTKs^[7-13]. Upon activation of FAK, a number of focal adhesion components, such as paxillin, tensin, Shc and P130cas, are phosphorylated and activate the ERK2/MAPK signaling pathway network^[13-20]. Therefore, FAK may play a central role in integrin-stimulated signaling events^[20], but it is a precondition that integrins bind to the ECM and cluster in the plane of the cell membrane. Integrin expression has been studied^[21-34], but studies on the expression integrin $\alpha 5$ and $\beta 1$ subunits and FAK in gastric cancer and uterocervical cancer are rare. Furthermore, the correlation between integrin and FAK expression has not been studied, and the difference of integrin or FAK expression among these five kinds of cancer has not been reported either.

In the present study, the authors used an immunohistochemical technique to examine the expression of integrin $\alpha 5$ and $\beta 1$ subunits and FAK in gastric cancer, colorectal cancer, hepatocellular carcinomas, uterocervical cancer, and breast cancer. In addition, the authors correlated the expression of FAK and integrin $\alpha 5$, and $\beta 1$ subunits with clinicopathologic data, including tumor differentiation, infiltration and metastasis.

MATERIALS AND METHODS

Specimens

Tissue samples were obtained from 75 cases of gastric carcinoma (40 males and 35 females), 21 colorectal cancer (11 males and 10 females), 16 hepatocellular carcinomas (10 males and 6 females), 20 uterocervical cancer, and 20 breast cancer that had undergone total or partial resection in Tumor Hospital, Shanghai Medical University from Jan. 1995 to Jan. 1996. Patient age ranged from 20 to 85 years old. Of the specimens, 51 cases of gastric cancer, 16 colorectal cancer, 16 hepatocellular carcinomas, 18 uterocervical cancer and 17 breast cancer were with unaffected margins. The specimens were fixed in 4 % methanal solution, embedded in wax, and cut in 5 μ m serial sections.

Reagents

Antibodies used in this study were as follows: rabbit anti-mouse FAK polyclonal antibody, biotinylated sheep anti-rabbit IgG, and biotinylated sheep anti-mouse IgG from Santa Cruz Biotechnology, Inc. (California, U.S.A.). Monoclonal mouse antibodies directed against human integrin $\alpha 5$ subunit and human integrin $\beta 1$ subunit were provided by Dr. Mingzhe Zheng at University of Washington, Seattle, U.S.A

Immunohistochemistry

Immunohistochemical staining was performed using the avidin-biotin-peroxidase technique^[35]. Anti-FAK antibody was diluted into 1:100, anti- $\alpha 5$ 1:15, and anti- $\beta 1$ 1:80. The avidin-biotin-peroxidase reagent kit was purchased from Vector Laboratories Inc.

A semiquantitative evaluation system was used to determine antigen expression in tissue samples^[36]. Expression was graded on the following scale: negative reaction, (-); mild positive, (+); moderate positive, (++) or strong positive, (+++). Evaluation of cell-surface and cytoplasmic staining was performed independently by two of the authors. In the occasional instance of disagreement, the slide was reviewed by the third observer, and a consensus opinion was obtained. The protein expression was correlated with tumor grades, which was in accordance with WHO classification.

Statistical analysis

was performed by using the χ^2 test. A *P* value of <0.05 was considered significant.

RESULTS

Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human gastric carcinomas

The expression patterns of the integrin subunits $\alpha 5$ and $\beta 1$ and FAK in the 75 cases of gastric carcinomas and 51 unaffected margins are summarized in Table 1. Of the 51 unaffected margin specimens, only 2 (4 %) showed moderate (++) FAK, others showed either negative or minimal FAK, but 39 (76 %) cases showed moderate (++) or strong (+++) integrin $\alpha 5$ immunoreactivity (Figure 1 A), and 43 (85 %) showed moderate (++) or strong (+++) integrin $\beta 1$ immunoreactivity. Of the 75 cancer specimens, 43 (57 %) samples showed moderate (++) or strong (+++) FAK immunoreactivity (Figure 3 A), 22 (30 %) samples had moderate integrin $\alpha 5$ immunoreactivity, and 19 (25 %) showed moderate integrin immunoreactivity. None of 75 cancer specimens showed strong immunoreactivity (+++) and neither integrin $\alpha 5$ nor $\beta 1$ subunit. In contrast with 51 unaffected margin specimens, 35 (69 %) carcinoma specimens had reduced $\alpha 5$ immunoreactivity, 43 (85 %) had reduced $\beta 1$ immunoreactivity, and 46 (90 %) had increased FAK immunoreactivity. So a significant difference was found between gastric carcinomas and their unaffected margins in the expression of FAK, integrin $\alpha 5$, and $\beta 1$ subunit.

When FAK expression was compared with histopathological and clinical parameters, the authors found that 30 (68 %) of 44 cases of poorly-differentiated cancer showed moderate or strong immunoreactivity, however, only 13 (42 %) of 31 well-differentiated cancer showed moderate or strong; Of the 59 deep or full stratum invasive cancer, 40 (68 %) showed moderate or strong, and only 3 (23 %) of 16 superficial or mucous invasive cancer showed moderate or strong; 30 (77 %) of 39 cases with lymph node metastasis showed moderate or strong, but 13 (36 %) of 36 cases without lymph node metastasis showed moderate or strong activity. So, a significant association was found between enhanced expression of FAK and poor differentiation, deep invasion, and lymph node metastasis of gastric carcinomas.

In addition, of the 44 cases of poorly-differentiated cancer, 36 (81.8 %) showed negative or minimal $\alpha 5$ and 37 (84 %) showed negative or minimal $\beta 1$, of the 31 moderate or well differentiated cancers, 17 (55 %) showed negative or minimal $\alpha 5$ and 19 (61 %) showed negative or minimal $\beta 1$. Reduced expression of subunit $\alpha 5$ and $\beta 1$ was significantly associated with poorly-differentiated carcinomas, but not with invasion and lymph node metastasis.

Finally, the authors found that 30 of the 43 gastric

carcinomas with high FAK expression (++) or (+++) had over expression of integrin subunit $\alpha 5$ and $\beta 1$ in the tumor tissues and unaffected margin of the same specimens.

Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human colorectal carcinomas

The expression patterns of the integrin subunits $\alpha 5$ and $\beta 1$ and FAK in the 21 colorectal carcinomas and 16 unaffected margins are summarized in Table 2.

Table 1 Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human gastric carcinomas and their relation to clinical and histological variables

	FAK		Integrin $\alpha 5$		Integrin $\beta 1$	
	-/+	++/+++	-/+	++/+++	-/+	++/+++
Type of tissues						
Unaffected margin specimen	49	2	12	39	8	43
Gastric carcinoma	32	43	53	22	56	19
	<i>P</i> <0.01		<i>P</i> <0.01		<i>P</i> <0.01	
Differentiation						
Poor	14	30	36	8	37	7
Moderate or well	18	13	17	14	19	12
	<i>P</i> <0.05		<i>P</i> <0.05		<i>P</i> <0.01	
Extent of invasion						
Mucosa or superficial stratum	13	3	11	5	12	4
Deep or full stratum	19	40	42	17	44	15
	<i>P</i> <0.01		NS		NS	
Metastasis						
Absent	23	13	26	10	29	7
Present	19	30	27	12	27	12
	<i>P</i> <0.01		NS		NS	

NS: No significant

Table 2 Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human colorectal carcinomas and their relation to clinical and histological variables

	FAK		Integrin $\alpha 5$		Integrin $\beta 1$	
	-/+	++/+++	-/+	++/+++	-/+	++/+++
Type of tissues						
Unaffected margin specimen	15	1	8	8	5	11
Gastric carcinoma	8	13	17	4	13	8
	<i>P</i> < 0.01		<i>P</i> < 0.01		<i>P</i> < 0.01	
Differentiation						
Poor	2	9	9	2	7	4
Moderate or well	6	4	8	2	6	4
	<i>P</i> < 0.05		NS		NS	
Extent of invasion						
Mucosa or superficial stratum	4	0	3	1	2	2
Deep or full stratum	4	13	14	3	11	16
	<i>P</i> < 0.01		NS		NS	
Metastasis						
Absent	6	4	9	2	9	2
Present	2	9	8	2	4	6
	<i>P</i> < 0.01		NS		<i>P</i> <0.05	

NS: No significant

In contrast with 16 unaffected margin specimens, 10 (62 %) carcinoma specimens had reduced $\alpha 5$ immunoreactivity (Figure 1 B), 6 (37 %) had reduced $\beta 1$ immunoreactivity (Figure 2A, 2B), and 13 (81 %) had increased FAK immunoreactivity (Figure 3 B). So a significant difference was found between colorectal carcinomas and their unaffected margins in the expression of FAK, integrin $\alpha 5$, and $\beta 1$ subunit.

When FAK expression was compared with histopathological and clinical parameters, A significant association was also found between over expression of FAK and poor differentiation, deep invasion, and present lymph node metastasis of colorectal carcinomas.

In addition, low expression of subunit $\alpha 5$ and $\beta 1$ was significantly associated with poorly-differentiated colorectal carcinomas, but not with invasion and metastasis.

Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human hepatocellular carcinomas

The expression patterns of the integrin subunits $\alpha 5$ and $\beta 1$ and FAK in the 16 hepatocellular carcinomas and their unaffected margins are summarized in Table 3.

Table 3 Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human hepatocellular carcinomas and their relation to clinical and histological variables

	FAK		Integrin $\alpha 5$		Integrin $\beta 1$	
	-/+	++/+++	-/+	++/+++	-/+	++/+++
Type of tissues						
Unaffected margin specimen	15	1	3	13	1	15
Hepatocellular carcinoma	4	12	11	5	10	6
	$P < 0.01$		$P < 0.01$		$P < 0.01$	
Differentiation						
Poor	1	7	8	0	7	1
Moderate or well	2	5	3	5	4	5
	$P < 0.05$		$P < 0.01$		$P < 0.05$	

In contrast with 16 unaffected margin specimens, 11 (69 %) carcinoma specimens had reduced $\alpha 5$ immunoreactivity, 7 (44 %) had reduced $\beta 1$ immunoreactivity, and 11 (69 %) had increased FAK immunoreactivity. So a significant difference was found between hepatocellular carcinomas and their unaffected margins in the expression of FAK, integrin $\alpha 5$ and $\beta 1$ subunit.

When these protein expression was compared with the degree of differentiation the authors found a significant association between these protein expression of FAK and poor differentiation in hepatocellular carcinomas.

Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human uterocervical carcinomas

The expression patterns of the integrin subunits $\alpha 5$ and $\beta 1$ and FAK in the 20 uterocervical carcinomas and 18 unaffected margins are summarized in Table 4. Comparing with 18 unaffected margin specimens, 13 (72 %) carcinoma specimens had reduced $\alpha 5$ immunoreactivity, 14 (78 %) had reduced $\beta 1$ immunoreactivity, and 3 (17 %) had increased FAK immunoreactivity. So the positive percentage of integrin $\alpha 5$ or $\beta 1$ had a significant difference between carcinomas and their unaffected margins. That of FAK was not apparent.

A significant association was not found between increased expression of FAK and poor differentiation or deep invasion of uterocervical carcinomas. Reduced expression of subunit $\alpha 5$ and $\beta 1$ was significantly associated with poor differentiated carcinomas, but not with invasion.

Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human breast carcinomas

The expression patterns of the integrin subunits $\alpha 5$ and $\beta 1$ and FAK in the 20 breast carcinomas and 17 unaffected margins are summarized in Table 4. Similarly, the increased FAK expression was significantly associated with breast carcinogenesis and lymph node metastasis of breast carcinomas. Reduced expression of subunit $\alpha 5$ and $\beta 1$ was significantly associated with breast carcinogenesis and poorly-differentiated carcinomas, but not with lymph node metastasis. In contrast with 17 unaffected margin specimens, 14 (82 %) carcinoma specimens had reduced $\alpha 5$ immunoreactivity, 16 (94 %) had reduced $\beta 1$ immunoreactivity, and 14 (82 %) had increased FAK immunoreactivity.

Table 4 Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human uterocervical carcinomas and their relation to clinical and histological variables

	FAK		Integrin $\alpha 5$		Integrin $\beta 1$	
	-/+	++/+++	-/+	++/+++	-/+	++/+++
Type of tissues						
Unaffected margin specimen	18	0	13	5	9	9
adenocarcinoma	9	1	9	9	1	9
Squamous carcinoma	10	0	10	0	9	1
	$P > 0.05$		$P > 0.05$		$P > 0.05$	
Differentiation						
Poor	9	1	10	0	9	1
Moderate or well	10	0	9	1	9	1
	$P > 0.05$		$P > 0.05$		$P > 0.05$	
Extent of invasion						
Mucosa or superficial stratum	5	0	5	0	4	1
Deep or full stratum	14	1	14	1	14	1
	$P > 0.05$		NS		NS	

NS: No significant

Table 5 Expression of FAK and integrin subunits $\alpha 5$ and $\beta 1$ in human breast carcinomas and their relation to clinical and histological variables

	FAK		Integrin $\alpha 5$		Integrin $\beta 1$	
	-/+	++/+++	-/+	++/+++	-/+	++/+++
Type of tissues						
Unaffected margin specimen	17	0	8	9	6	11
Breast carcinoma	10	10	20	0	20	0
	$P < 0.01$		$P < 0.05$		$P < 0.01$	
Metastasis						
Absent	9	3	12	0	12	0
Present	1	7	8	0	8	0
	$P < 0.01$		NS		$P > 0.05$	

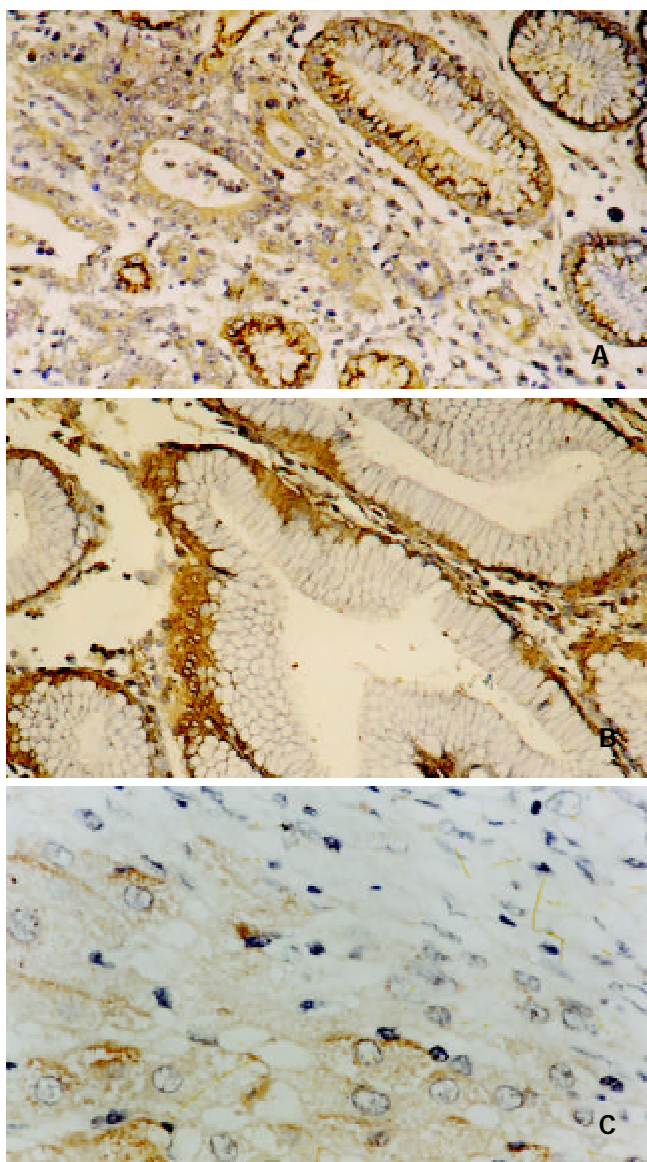


Figure 1 Integrin $\alpha 5$ subunit expressed in carcinoma and unaffected margin tissues, ABC-DAB, $\times 400$

A. Positive expression of integrin $\alpha 5$ subunit in unaffected margin tissues of gastric carcinoma.
B. Positive expression of integrin $\alpha 5$ subunit in unaffected margin tissues of colorectal carcinoma.
C. Positive expression of integrin $\alpha 5$ subunit in unaffected margin tissues of hepatocellular carcinoma.



Figure 2 A. Positive expression of integrin $\beta 1$ subunit in unaffected margin tissues of colorectal carcinoma, ABC-DAB, $\times 100$.

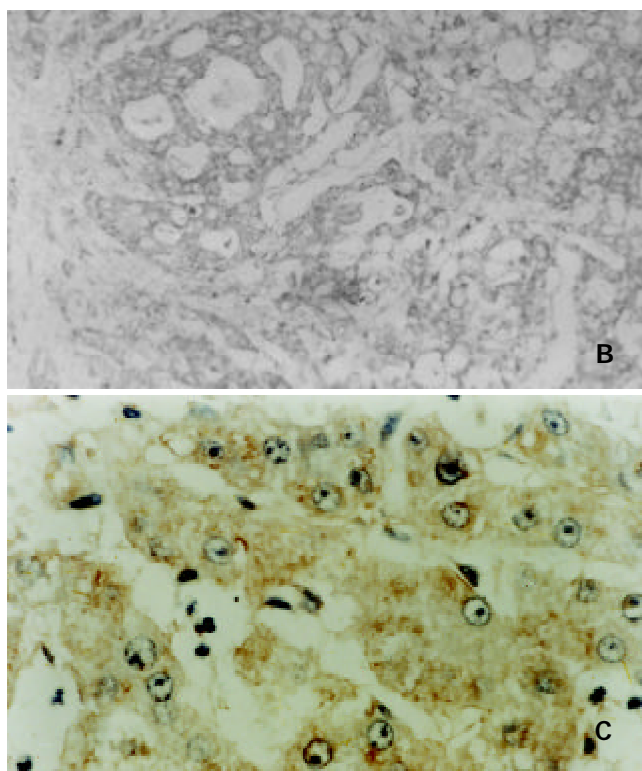


Figure 2 Integrin $\beta 1$ subunit expressed in carcinoma and unaffected margin tissues

B. Negative expression of Integrin $\beta 1$ subunit in colorectal carcinoma specimen, ABC-DAB, $\times 200$.
C. Positive expression of integrin $\beta 1$ subunit in unaffected margin tissues of hepatocellular carcinoma, ABC-DAB, $\times 400$.

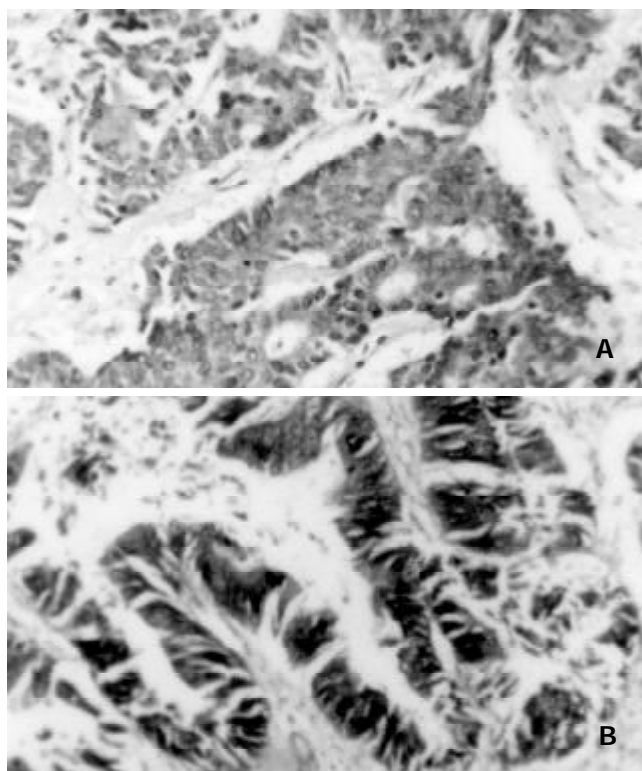


Figure 3 FAK expressed in carcinoma tissues, ABC-DAB, $\times 400$

A. Positive expression of FAK in gastric carcinoma.
B. Positive expression of FAK in colorectal carcinoma.

NS: No significant

DISCUSSION

This study indicated that the expression of the FAK antigen was lower in unaffected noncancerous margin tissues than in carcinoma tissues, whereas expression of integrin $\alpha 5$ and $\beta 1$ subunits was higher in unaffected margin than in carcinoma tissues. The quantity of all three proteins related to the degree of differentiation of the cancer. The reasons for these findings are complex. First, the contents of fibronectin and laminin in the ECM is lower in normal tissues than in cancerous counterparts. However, the fibroblast is stimulated by cancer cells to produce more matrix proteins thereby increasing the concentration of matrix proteins^[37]. Second, the increased expression of integrin $\alpha 5$ and $\beta 1$ subunits in preliminary cancer tissue make the matrix proteins relatively much more deficient. Under these conditions, FAK can exist in a state with high tyrosine-phosphorylated on the Tyr-397/Src SH2 binding site^[30-42]. However, the Src-family PTKs are not significantly associated with FAK that has only low levels of kinase activity and its expression is reduced^[13, 20, 43-45]. In unaffected margin tissues, the negative regulators of integrin-stimulated signaling events include the FAK-associated PTP, PTEN and the p130cas that all reduced the kinase activity of FAK^[46-48]. The quantity of matrix proteins is increased in the ECM of cancerous tissue. Integrins bind to the ECM, and become clustered in the plane of the cell membrane where they increase FAK tyrosine phosphorylation and kinase activity. Src-PTKs can significantly associate with FAK. Since FAK kinase activity is important for the FAK-enhanced increase in Src-PTK activity^[49] and Src-PTKs can also phosphorylate FAK within the kinase activation loop (Tyr-576 and Tyr-577) to promote maximal FAK kinase activity, the transient complex formed between FAK and Src after integrin stimulation of cells may lead to the maximal activation of both PTKs^[38, 50]. The survival signals are continually magnified, and cells with overexpression of FAK can inhibit the effects of PTEN on PI3-K activity and partially inhibit its effects on PIP₃ levels, Akt phosphorylation and cell apoptosis^[51]. Cells proliferate extensively. Therefore the authors believe overexpression of FAK is required for carcinogenesis. The reduced expression of integrin $\alpha 5, \beta 1$ subunit in carcinomas does not decrease the FAK kinase activity. Ca²⁺, PKC and Phorbol 12-myristate 13-acetate (PMA), can also increase FAK kinase activity^[20].

This study indicates that the relationship between integrin $\alpha 5, \beta 1$ subunits and metastasis is not significant in cancer cells, whereas FAK expression is significantly associated with metastasis or invasiveness. The process of cancer invasion or metastasis is complex, and involves attachment of the cancer cell to the ECM, decomposition of the ECM by proteases, and migration of cancer cells. The integrin subunits that aid cell adhesion are reduced in cancerous tissue, but integrin subunits that relate to cell migration are increased or constant^[52]. On the basis of these findings, the expression of a single integrin subunit may not correlate with metastasis, but FAK is different because it can associate with integrin $\beta 2, \beta 3$ and some α subunits^[20, 53]. Several other cellular stimuli that generate signals through either G-protein linked receptors, transmembrane growth factor receptors or other unknown mechanisms can increase the level of FAK tyrosine phosphorylation in cells^[20]. Thus, FAK may play a central role in signaling events stimulated by integrin or other molecules.

This study also indicates that the quantity change of integrin $\alpha 5, \beta 1$ and their related signal molecule FAK is relate to the type of cancer. For integrin $\alpha 5$, breast carcinoma (82 %) had the most reduced expression, then uterocervical carcinoma

(72%), gastric carcinoma (69 %), hepatocellular carcinoma (69 %), colorectal carcinoma (62 %) successively. For integrin $\alpha 1$, breast carcinoma (94 %) also had the most reduced expression, then gastric carcinoma (85 %), uterocervical carcinoma (78 %), hepatocellular carcinoma (44 %), colorectal carcinoma (37 %) successively. For FAK, gastric carcinoma (90 %) had the most increased expression, then colorectal carcinoma (80 %), breast carcinoma (80 %), hepatocellular carcinoma (69 %), uterocervical carcinoma (37 %) successively. From the above data, the authors find that the changes of integrin $\alpha 1$ and FAK is much more related with the type of cancer.

In summary, the authors have shown in the present study that expression of FAK is more significantly associated with carcinogenesis, differentiation and metastasis than that of integrin $\alpha 5$ and $\beta 1$ subunits. FAK may be not only a transformation-linked enzyme but also a progression-linked enzyme. The level of FAK expression might be a valuable marker for the carcinogenesis and progression of some types of carcinoma. The expression of integrin $\alpha 5, \beta 1$ and FAK is relate to the type of cancer.

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