THE IMPLICATION OF CORTACTIN OVEREXPRESSION IN COLORECTAL CANCER

(cortactin 過量表達與大腸直腸癌的關係)

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主持人：馬明琪　　私立中山醫學院生化所

一、中文摘要

為探討 colorectal cancer 是如何形成的，本實驗室和成功大學醫學院呂增宏老師、李政昌醫師合作，分析了 56 位大腸癌患者其正常及癌症的檢體。我們發現大腸癌檢體 FAK 及 c-Src 過量表達的比率分別是 89.5 % 及 94.64 % ，且兩者在 colon cancer 的增加似乎有同步的傾向。而另外我們也在81% colon cancer 中發現了 cortactin 的過量表達。

關鍵詞：大腸癌、蛋白表達、FAK、c-Src、cortactin

Abstract

In order to investigate the tumorigeneic mechanisms of colon cancer, we collaborated with Drs. Lee, J.-C., and Leu, T.-H. to analyze 56 paired cancer-normal mucosa specimens from colorectal cancer patients. Compared to normal mucosa, enhanced FAK and c-Src expression in tumor specimens (T/N > 1) was observed in 89.5% and 94.64% tumor samples respectively. Interestingly, we observed paralleled enhancement of protein expression of both nonreceptor tyrosine kinases in colorectal cancer. Furthermore, enhanced cortactin overexpression was also detected in 81 % colon tumor specimens.

Keywords: colorectal cancer、protein expression、FAK、c-Src、cortactin

二、緣由與目的

As the most common gastrointestinal cancer, colon carcinoma is one of the leading causes of cancer mortality worldwide (1). At least 15 % of colorectal cancers was reported to occur in dominantly inherited pattern, and FAP (familial adenomatous polyposis) and HNPCC (hereditary nonpolyposis colorectal cancer) were the two best defined familial forms (2). While the former affected tumor initiation by targeting the *APC* gene, the latter affected tumor progression by targeting genes involved in mismatch repair (MMR). The genetic bases for both of these syndromes have been intensively studied, and at least 7 genetic events have been identified to underlie the development of colorectal cancer (2).

The proto-oncogene product, c-Src, is a nonreceptor tyrosine kinase (3). Previous studies indicated that c-Src was required for both EGF-induced mitogenesis in fibroblasts (4) and EGF-dependent migration in epithelial cells (5). In addition, c-Src is also involved in cell movement in endothelial cells (6). The synergistic increase of oncogenic capacity of EGF receptor (EGFR) by c-Src overexpression implicates the interaction between these two tyrosine kinases that may contribute to a more aggressive phenotype in human tumor (7). Indeed, in colon cancer, c-Src can interact with multiple receptor tyrosine kinases including EGFR in a kinase-dependent

fashion that can promote the metastatic potential of the tumor (8).

As initially identified as a v-Src substrate (9), focal adhesion kinase (FAK), turned out to be involved in cell-matrix interaction and integrin signaling (10). While its expression and phosphorylation can influence cell migration in epithelial cells (11), impaired cell mobility is observed in fibroblasts devoid of FAK (12). In colon cancer, the correlation between enhanced FAK expression and increased invasive potential was reported (13).

In response to integrin engagement, FAK becomes activated and autophosphorylated. Tyr-397 is the major FAK autophosphorylation site whose phosphorylation confers the binding site for Src family kinases (14, 15). The interaction between these two kinases accelerates Src-mediated FAK phosphorylation. Among the Src-mediated sites on FAK, Tyr-576/577 phosphorylation can enhance the enzymatic activity of FAK (16, 17) while Tyr-925 phosphorylation can associate with Grb2 leading to Ras activation and triggers MAP kinase cascade (18, 19).

Compared to the well-studied genetic alterations in colon cancer, the involvement of tyrosine kinases in this disease is still obscure. Though both c-Src and FAK are implicated in colorectal cancer and the activity of FAK is modulated by c-Src, to date, no study analyzing these two kinases simultaneously has been reported. Here, we sought to explore the relation between the expression of c-Src and FAK, and the clinico-pathological features in a group of 56 colorectal carcinomas. Surprisingly, we found paralleled enhancement of both protein expression in colorectal cancer and compared to peritoneal seeding, the expression of these two nonreceptor tyrosine kinases and the level of paxillin was further enhanced in liver metastatic colon tumor.

三、結果與討論

Enhanced kinase activities detected in many human colon cancers. Both Tyr- and Ser/Thr kinases are important in the regulation of normal cellular functions. Their abnormal expression and/or activation often lead to cellular transformation. To identify the important players in human colon carcinoma, we looked for the kinase(s) that possessed above-than-normal activity. To address this question, in situ gel kinase assays of paired normal mucosa and tumor specimens were carried out (Figure 1 and data not shown). In the presence of poly(Glu-Tyr) in the gel, proteins with molecular weights of 125-, 100-, 85-, 60-, 50-, 45-, and 35 kDa exhibited enhanced trans-phosphorylation activity in tumor specimens as compared to that detected in a representative normal mucosa (Figure 1A). In other words, these tyrosine kinases were activated in colon cancer with increased capacity to phosphorylate poly(Glu-Tyr). By contrast, when poly(Glu-Tyr) was not embedded in polyacrylamide gel, proteins with molecular weights of 125-, 60-, 45 kDa showed increased autophosphorylation activity in tumor specimens as compared to that detected in normal mucosa. These data indicated that these proteins could phosphorylate themselves and could be Tyr- or Ser/Thr kinases. According to the molecular weights of known tyrosine kinases, the 60- and 125-kDa proteins were likely to correspond to c-Src and FAK respectively.

And interestingly, there are reports demonstrating the elevated expression of c-Src (8), FAK (13) and the enhanced kinase activity of c-Src (8, 21, 22) in colon cancer. As the potential kinases with enhanced enzymatic activity in colon tumor specimens, the expression of c-Src and FAK was simultaneously analyzed in this study.

The expression levels of FAK in fresh human tissue specimens. Human tissue samples derived from patients with colorectal cancer were subjected to Western blot analysis of FAK levels. Figure 2A showed the results of immunoblot analysis of 7 representative paired normal mucosa and tumors with distinct expression pattern of FAK. The FAK amounts in tumor specimens were significantly increased as compared to those detected in paired normal mucosa. And identical sets of lysates were further immunoblotted with antibody against actin to exclude potential errors introduced by differential protein loading (Fig. 2C). The expression of FAK was compared in 56 primary colon carcinomas. Overexpression of FAK in tumor was defined as the ratio of FAK amount in tumor to that in normal mucosa was greater than 1 (T/N ration > 1). Forty-nine of 56 specimens (89.5 %) exhibited FAK overexpression. Analysis of the association between FAK expression and other risk factors such as histological differentiation, Dukes' stage, sex, age, and tumor location indicated that none of these associations were statistically significant (Table 1).

The expression levels of c-Src in fresh human tissue specimens. To analyze c-Src levels, cell lysates prepared as described above were analyzed by Western immunoblotting. As shown in Figure 2B, compared to normal mucosa, the levels of c-Src in tumor specimens were increased. And among the seven representative patients analyzed, six exhibited the overexpression of c-Src (T/N ratio > 1). The relationship between c-Src expression and clinicopathological factors of the 56 patients analyzed was summarized in Table 1. Among the 56 patients, 94.6% (53/56) exhibited c-Src overexpression. And analysis of the association between c-Src expression and the risk factors including histological differentiation, Dukes' stage, sex, age and tumor location also indicated that none of these associations were statistically significant (Table 1). Thus, from the results described above, no significant associations were found between FAK, c-Src, and clinical parameters. In conclusion, both FAK and c-Src were not significant prognostic factors for patients' disease-free survival in short-term follow up.

Paralleled expression of FAK and c-Src in colorectal cancer. Since we found that both FAK and c-Src tended to overexpress in colorectal tumor specimens in this study, we further utilized Pearson product moment to calculate the correlation between FAK and c-Src. As shown in Figure 3, FAK was positively correlated to the expression of c-Src ($r = 0.51$; $p < 0.0001$).

Increased FAK and c-Src expression in liver-metastatic colorectal tumor but not in peritoneal metastasis colon cancer. From the data described above, FAK and c-

Src turned out to be the non-prognostic factors for colon patients' disease-free survival. However, FAK and c-Src have been speculated in tumor metastasis, therefore, we were interested in dissecting the involvement of FAK and c-Src during the progression of colorectal tumor to metastatic disease. Among all the patients analyzed in this study only three underwent tumor resection at both colon and metastatic tissue(s) at the same time. Patient No. 4 had peritoneal metastasis while patient No. 18 and 55 had liver metastasis. The expression of FAK and c-Src in all the specimens derived from colon cancer and their paired normal mucosa and tumors at the metastatic tissues was further analyzed. To exclude the possibility of differential protein loading in all the specimens analyzed, the expression of actin was utilized as an internal control. And as shown in the bottom panel in Fig. 4, all specimens analyzed contained similar amounts of proteins. In relative to actin, fairly increased amount of FAK and c-Src was detected in the liver-metastatic sample as compared to primary colorectal tumor. In contrast, such phenomenon was not detected in peritoneal metastasis colon cancer (Figure 4). Interestingly, when the levels of the focal contact protein paxillin were analyzed in all these samples, similar expression pattern as that described in FAK was observed (Figure 4). Thus, the expression of FAK, paxillin and c-Src seemed to correlate with colon cancer with liver metastatic potential. Finally, the overexpression of cortactin was observed in 81 % colon cancer specimens examined (Fig. 5).

四、計畫成果自評

本計畫進行順利，目前已有一篇 manuscript 投稿、另一篇 manuscript 正在準備中。

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Table 1. Relationship between expression of FAK, c-Src, and clinicopathological features.

	FAK (T/N ratio)		P-Value	c-Src (T/N ratio)		P-value
	>1	≤1		>1	≤1	
	N=56			N=56		
Total No of cases	49	7		53	3	
Gender (M/F)	26/23	5/2	0.44	28/25	3/0	0.24
Age(years)	64.5±11.2	64.1±12.6	0.83	64.1±11.4	70.3±8.3	0.34
Location						
Rectum + Sigmod	36	6		39	3	
Proximal	13	1	0.67	14	0	0.57
Histological Differentiation						
Well	16	2		16	2	
Moderately	30	4		33	1	
Poorly	3	1	0.65	4	0	0.42
Dukes Classification						
A	2	0		2	0	
B	24	2		25	1	
C	17	5		20	2	
D	6	0	0.42	6	0	0.74
Liver Metastasis						
H (-)	38	6		41	3	
H (+)	11	1	1.0	12	0	1.0
Distant metastasis						
No	32	5		22	3	
Yes	17	2	1.0	19	0	0.25

All the continuous data were expressed as Mean ± SD.

Generalized Fisher's exact test was used to compare categorical data and Wilcoxon rank sum test to compare continuous data.

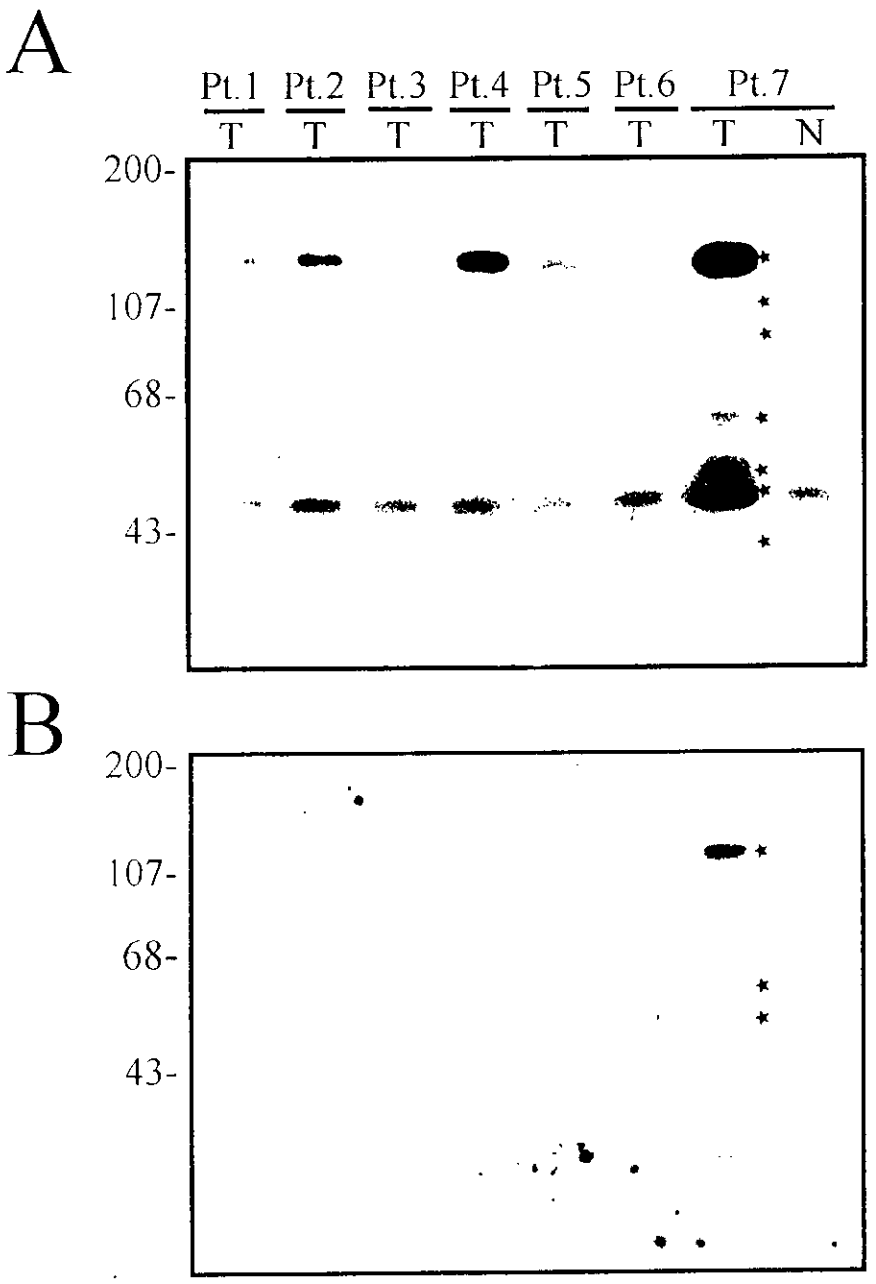

Fig. 1. Detection of the activated kinases in colon tumor specimens. Tumor specimens (T) and one of their normal counterparts (N) derived from colon cancer patients were loaded onto poly (Glu-Tyr)-embedded SDS/PAGE (A) or SDS/PAGE (B). Then the in situ gel kinase assays were carried out as described in MATERIALS AND METHODS. The positions of various proteins with enhanced transphosphorylation (A) and autophosphorylation (B) in tumor lysates of patient No. 7 are marked with asterisks.

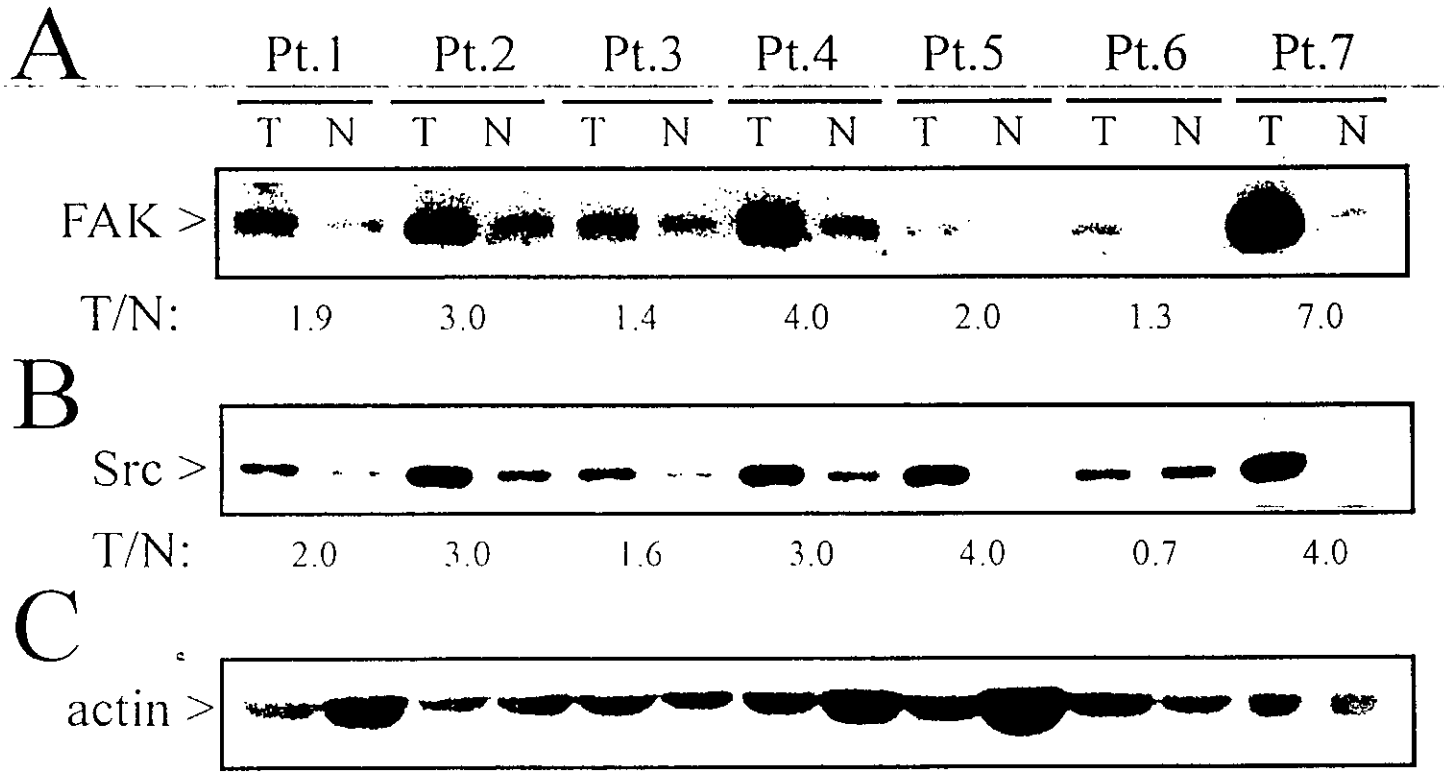

Fig. 2. The expression of FAK and c-Src in colon tumor specimens. The expression of FAK (A) and c-Src (B) of seven representative patients was revealed by Western immunoblotting with respective antibodies and quantified by densitometer scanning. T: tumor tissue; N: normal tissue. The ratios of FAK and c-Src expression between tumor and normal tissue (T/N) of each patient were calculated and demonstrated. Meanwhile, the actin expression in all these samples was demonstrated by immunoblotting with actin antibody.

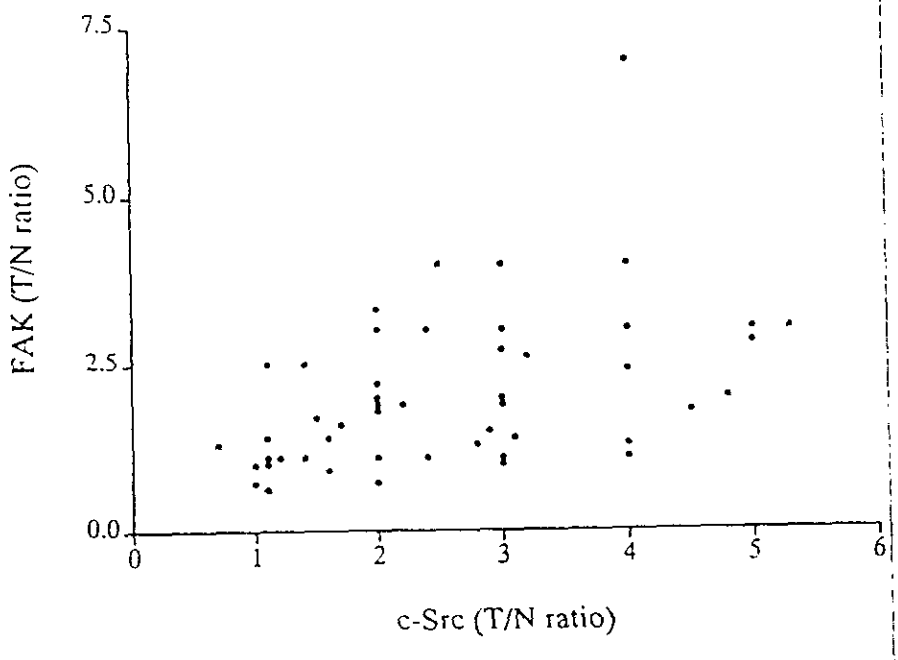

Fig. 3. The scatterplot of FAK against c-Src (r = 0.51, 95 % confidence interval: 0.28– 0.68).

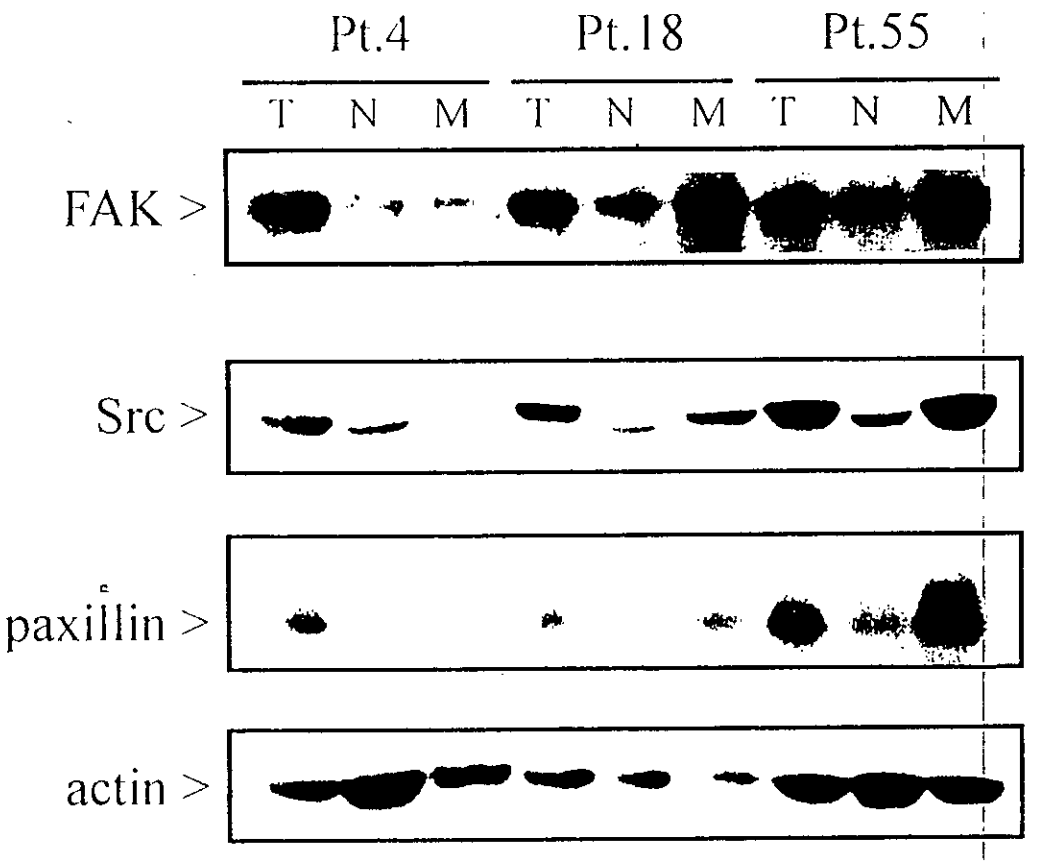

Fig. 4. The expression of FAK, c-Src, and paxillin in patients with synchronous primary and metastatic colorectal tumors. The expression of FAK, c-Src, and paxillin in patients with peritoneal colon cancer (patient no. 4) and liver metastatic colon tumor (patient no. 18 and 55) was revealed by Western immunoblotting with respective antibodies. T: tumor tissue; N: normal tissue; M: metastatic tumor tissue. The expression of actin in all samples was utilized as an internal control.

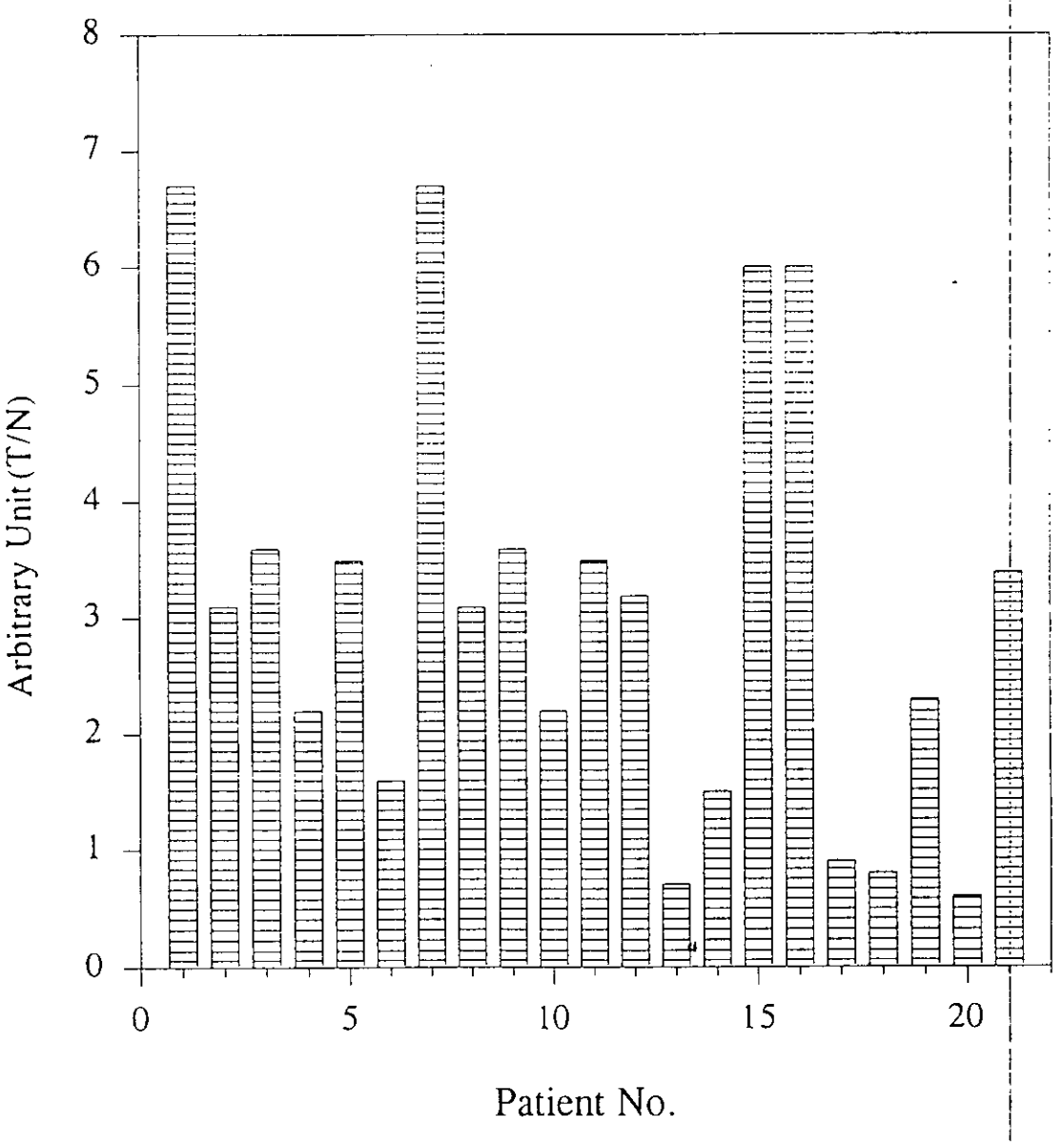

Fig. 5. Overexpression of cortactin in Taiwanese colon cancer. The ratio of cortactin expression between tumor and normal tissues was quantified by densitometer scanning and the number of cortactin in normal tissues was arbitrarily assumed to be one. Overexpression pattern of these proteins in 21 patients was shown in this histography.