Pergamon

European Journal of Cancer Vol. 32A, No. 8, pp. 1401–1407, 1996

Printed in Great Britain
0959-8049/96 $15.00+0.00

PII: S0959-8049(96)00090-1

Original Paper

Augmented Expression of *LCK* Message Directed From the Downstream Promoter in Human Colorectal Cancer Specimens

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Lck protein is expressed in some colon carcinoma cell lines but its expression in colon cancer cells *in vivo* has not been clarified. *LCK* transcription is regulated from two distinct promoters and initiated exclusively from the downstream promoter in colon carcinoma cell lines in contrast to peripheral lymphocytes. We investigated the expression of the downstream promoter-initiated *LCK* transcript in 18 colorectal primary cancer and normal mucosae, and two hepatic metastases, using a RNase protection assay with the *Eco*RI–*Bgl*II fragment of human *LCK* cDNA, YT16. In normal tissues, only traces of the LCK transcript were detected. The expression of the *LCK* transcript was augmented in 3/18 cancer specimens. The relative level of the *LCK* transcript in the cancer tissue compared to the average value of normal adjacent tissue was 10–60 in 3 cases, and 3–10 in 7 cases. One hepatic metastasis expressed more *LCK* message than the primary lesion. Our results indicate that the *LCK* message is strongly expressed in some colorectal cancers.

Key words: protein-tyrosine kinase, colorectal neoplasms
Eur J Cancer, Vol. 32A, No. 8, pp. 1401–1407, 1996

INTRODUCTION

MULTIPLE GENETIC abnormalities have been implicated in the carcinogenesis of human colorectal cancer [1]. These alterations are divided into two major types: deletion or mutation of tumour suppressor genes, such as *P53* mutation which is frequently observed in colorectal cancer [2]; and activation of proto-oncogenes, such as mutation of *C-RAS* [3]. Although each abnormality occurs with some frequency in colorectal tumours, none is sufficient for the carcinogenesis of colorectal cancer by itself. As alterations in four or five genes simultaneously are usually observed in a colorectal cancer, sequential steps of genetic alterations are proposed as a model of colorectal tumorigenesis.

Protein tyrosine kinases transduce signals which are essential to cell growth [4]. *SRC*-family protein tyrosine kinases which include $p60^{c\text{-}src}$, $p61^{c\text{-}yes}$, $p56^{lck}$, $p59^{fyn}$, $p59^{hck}$, $p56^{lyn}$, $p55^{c\text{-}fgr}$ and $p55^{blk}$, are non-receptor type and share common structures. The conserved features of this family of kinases are (i) association with the inner cell membrane through myristylation of the amino-terminal glycine residue; (ii) three homologous sequences termed *SRC*-homology domains including a tyrosine kinase domain; and (iii) a tyrosine residue in the carboxyl-terminal, which is phosphorylated *in vivo* and an important regulatory site for their kinase activity [5, 6]. The amino-terminal domain is unique for each member. It has been demonstrated that some src-related kinases have potential oncogenic activity [7–11]. Kinase activity of $p60^{c\text{-}src}$ and $p61^{c\text{-}yes}$ is frequently increased in human colorectal carcinoma cell lines and cancer tissues [12–16]. Pharmacological inhibition of protein tyrosine kinase activity causes significant reduction of cell proliferation of src-activated colon cancer cell lines [17], suggesting that src-related proteins play an important role for aberrant cell growth of cancer cells. Recently, it has been demonstrated that src activity is greater in metastases of colorectal cancer than in primary lesions, which suggests that src kinase is involved in the progression of colorectal cancer [18, 19].

Another member, $p56^{lck}$, is expressed predominantly in lymphoid tissue, physiologically [20]. Lck is expressed in some human colon carcinoma cell lines [21], but it is not yet precisely evaluated whether lck is expressed in colorectal cancer cells and normal colonic epithelia *in vivo*. The residence of lymphocytes in colonic mucosa makes it difficult to

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Received 4 Jul. 1995; revised 15 Jan. 1996; accepted 16 Jan. 1996.

examine its expression in colonic tissues. As a detected signal of lck could be expressed either in cancer cells or in lymphocytes, conventional Northern or Western blot analysis is unable to discriminate its origin. Transcription of the *LCK* gene is regulated by two distinct promoter elements which direct the expression of two kinds of *LCK* mRNA transcripts that differ only within their 5′ untranslated regions [22]. Peripheral blood T-lymphocytes contain only *LCK* transcript initiated from the upstream promoter, whereas thymocytes express two types of *LCK* message directed from both the upstream and the downstream promoter [23]. Aberrant expression of *LCK* transcript in colorectal cancer cell lines is directed exclusively from the downstream promoter [23, 24]. Although these observations suggest that abnormal expression of $p56^{lck}$ is concerned with the development of colorectal cancer, we can not exclude the possibility that this molecular event occurs during the establishment of cancer cell lines *in vitro* or lck is expressed only in minor populations of cancer cells *in vivo*. It has not been demonstrated whether $p56^{lck}$ is expressed in colorectal cancer specimens, and if it is expressed, the clinical relevance of the percentage of the *LCK*-expressing tumour. In this report, we demonstrate that the downstream promoter-directed *LCK* mRNA is scarcely expressed in normal colorectal tissues and is overexpressed in some colorectal cancer specimens in comparison with that in adjacent normal mucosa.

MATERIALS AND METHODS

Cells and colonic tissues

Human colon carcinoma cell lines Colo201 and WiDr were obtained from the American Type Culture Collection (Rockville, Maryland, U.S.A.) and Japanese Cancer Research Resources Bank (Tokyo, Japan), respectively. Jurkat and U937 are a human leukaemic T-cell and a monocytoid cell line, respectively, and their characters have been described elsewhere [23]. These cell lines were cultured at 37°C in RPMI 1640 medium (Gibco, Grand Island, New York, U.S.A.) supplemented with 10% fetal calf serum (Mitsubishi Kasei, Tokyo, Japan) in humidified air containing 5% CO_2. Primary colorectal cancer, colorectal cancer metastases and normal colorectal mucosal samples were obtained from surgically resected specimens collected at Kyushu University Hospital and Fukuoka Saiseikai General Hospital, and frozen in liquid nitrogen within 20 min of resection. In all cases, the diagnosis of primary colorectal cancer was made by pathologists. Patients' age and sex, locations of the tumours and stages according to the Astler–Coller classification are summarised in Table 1 for specimens used in the RNase protection assay. Five other colorectal cancer specimens were used for the Western blot analysis (pts A–E).

Reagents

Deoxycholate, aprotinin, leupeptin and RNase A were obtained from Sigma (St Louis, Missouri, U.S.A.), Triton X-100 and PIPES from Wako Pure Chemical (Osaka, Japan), Block Ace from Dainihon (Osaka, Japan), VECTASTAIN ABC kit from Vector Laboratories (Burlingame, California, U.S.A.), Alkaline Phosphatase Conjugate Substrate kit from Bio Rad (Hercules, California, U.S.A.), ISOGEN™ from Nippon Gene (Tokyo, Japan), SP6 Refill System and proteinase K from Promega (Madison, Wisconsin, U.S.A.), [α-^{32}P]-CTP from Amersham (Little Chalfont, U.K.) and formamide from Katayama Chemical (Osaka, Japan).

Table 1. List of individual tumours

Patient	Age	Sex	Location	Stage*	N†	Metastasis
1	55	M	R	B_1	(−)	(−)
2	81	F	R	B_1	(−)	(−)
3	53	M	R	B_1	(−)	(−)
4	43	F	R	D	(+)	(+)‡
5	51	F	S	B_2	(−)	(−)
6	63	M	S	D	(+)	(+)‡
7	63	M	R	D	(+)	(+)‡
8	52	M	S	B_2	(−)	(−)
9	63	M	As	C_2	(+)	(−)
10	60	F	S	B_1	(−)	( )
11	73	F	R	C_2	(+)	(−)
12	58	M	S	C_1	(−)	(−)
13	61	M	R	A	(−)	(−)
14	80	M	R	C_2	(+)	(−)
15	63	M	S	B_2	(−)	(−)
16	71	F	As	D	(+)	(+)§
17	64	M	R	B_2	(−)	(−)
18	72	M	S	B_2	(−)	(−)

*Stages were determined according to the Astler–Coller classification. †Lymph node metastasis. ‡Hepatic metastasis. §Peritoneal metastasis. M, male; F, female; R, rectum; S, sigmoid colon; As, ascending colon.

Antibody and immunoblot analysis

The specificity of anti-lck monoclonal antibody, MOL171, has been described previously [25, 26]. Cells and tissues were solubilised in RIPA buffer (150 mM NaCl/0.5% deoxycholate/1% Triton X-100/0.05% SDS/20 mM Tris–HCl pH 7.5) supplemented with protease inhibitors (final concentrations, aprotinin 10 μg/ml, leupeptin 10 μg/ml) on ice for 30 min and postnuclear supernatant was collected by centrifugation at 12000*g* for 20 min at 4°C. Protein (180 μg) was separated on 10% SDS–PAGE and transferred on to a nitrocellulose filter (Schleicher & Schuell, Dassel, Germany). The filter was pretreated with a blocking reagent, Block Ace, for 90 min at room temperature, prior to the incubation with MOL171 at the concentration of 0.8 μg/ml for 1 h at room temperature. Subsequently, the detection of the target protein was performed using VECTASTAIN ABC kit and Alkaline Phosphatase Conjugate Substrate kit according to the manufacturer's instructions. In brief, the filter was washed and treated with biotinylated antimouse IgG followed by incubation with avidin-biotinylated alkaline phosphatase. After washing, the filter was immersed in the substrate solution until the colour development had been completed. Densitometric analysis was performed using a Densito-pattern analyser, EPA-3000 (Chemiway, Tokyo, Japan).

RNA preparation

Total cellular RNA was isolated from cells and tissues in the presence of ISOGEN™, which is a mixture of phenol and guanidine thiocyanate [27].

Northern blot analysis

Northern blot analysis was performed as described previously [23]. [^{32}P]-random-primed labelled probe for β-actin message was generated from human β-actin cDNA which was purchased from Wako.

RNase protection assay

Basically, the RNase protection assay for *LCK* message was performed as described previously [23] with some minor

modifications. An *Eco*RI–*Bgl*II fragment of human *LCK* cDNA, YT16 [28], was cloned into pSPT18 (Boehringer, Mannheim, Germany), a plasmid vector which contains the transcriptional promoter for SP6 RNA polymerase. The constructed plasmid was then linearised using *Eco*RI, and the [^{32}P]-labelled antisense RNA probe, which is 362 base pairs in length, was synthesised using SP6 Refill System and [α-^{32}P]CTP. ^{32}P-labelled RNA probes (1×10^5 cpm) were hybridised with 90 μg of total cellular RNA in 30 μl of hybridisation buffer (80% formamide/0.4 M NaCl/1 mM EDTA/40 mM PIPES, pH 6.7) at 50°C for 12 h. After hybridisation, 300 μl of RNase A containing solution (40 μg/ml) in 300 mM NaCl/5 mM EDTA/10 mM Tris–HCl, pH 7.5, was added and incubated at 34°C for 30 min. The samples were then treated with proteinase K and SDS, followed by extraction with phenol/chloroform and ethanol precipitation. The precipitated RNA was dissolved in 3 μl of loading buffer, heated and loaded on a 6% polyacrylamide/8 M urea gel. Finally, the gel was dried and exposed to an X-ray film for 7 days. Densitometric analysis was performed using a Densito-pattern analyser, EPA-3000.

RESULTS

The amount of lck protein is increased in colorectal carcinoma

To determine whether the level of the lck protein is elevated in colorectal cancer, Western blot analysis, using the anti-lck monoclonal antibody MOL171, was performed on primary colorectal cancer and normal colorectal tissue specimens obtained from 5 patients (Figure 1). Jurkat and Colo201 are a leukaemic T-cell and a colon cancer cell line, respectively, known to express p56^{lck} abundantly and were used as positive controls. U937, a monocytoid cell line, does not contain p56^{lck} and was used as a negative control. A 56 kDa band was detected in Jurkat and Colo201 but not in U937. A 60 kDa protein was also detected in Jurkat and Colo201, which is a modified form of p56^{lck} with larger molecular weight [25, 29]. In normal colonic tissues, lck protein was detected at a very low level. In three cases, the amount of p56^{lck} in colorectal cancer tissue was more abundant than that found in normal mucosa adjacent to the tumour (patients B, C and D). Densitometric analysis revealed that the ratio of lck protein in cancer tissue to that in normal mucosa in patients B, C and D was 3.0, 9.4 and 2.1, respectively. This finding suggests that lck protein is expressed in some colorectal cancer cells, but whether this signal is due to *LCK* expression in colonic normal epithelia and cancer cells or existence of lymphocytes in the stroma of colonic mucosae and tumors cannot be determined from this result.

The expression of the downstream promoter-directed LCK *transcript is augmented in colorectal carcinoma*

LCK gene contains two distinct promoter elements which direct the expression of *LCK* mRNAs that differ only within their 5′ untranslated regions [22]. In peripheral blood lymphocytes, only *LCK* message initiated from the upstream promoter is expressed [23]. In colon cancer cell lines which express lck, transcription of the *LCK* gene is directed exclusively from the downstream promoter [23, 24]. Thus, analysis of the promoter usage of the *LCK* gene will be useful to discriminate between aberrant expression in colon cancer cells and infiltration of lymphocytes into colorectal tumours. We performed a RNase protection assay of the *LCK* message with an antisense RNA probe derived from a *Eco*RI–*Bgl*II fragment of the human *LCK* cDNA, YT16 (Figure 2). This probe covered the 5′ untranslated region present in the upstream promoter-initiated *LCK* transcript as well as the downstream exons that are shared with *LCK* transcript initiated from the downstream promoter. As we reported previously [23], two kinds of protected fragments were observed with this probe. 325 base pair fragments represent the upstream promoter-initiated *LCK* transcript, and 238 base pairs fragments, the downstream promoter-directed one. Jurkat contained both types of *LCK* mRNA while Colo201, a colonic cancer cell line, contained only the downstream promoter-initiated one. WiDr, another colonic cancer cell line, did not reveal detectable message in contrast to Colo201. In U937, a monocytoid cell line, no detectable protected band was observed. In normal colonic tissues, traces of the downstream promoter-directed *LCK* message were observed. In colorectal cancer specimens from patients 2, 4 and 7, strong *LCK* message from

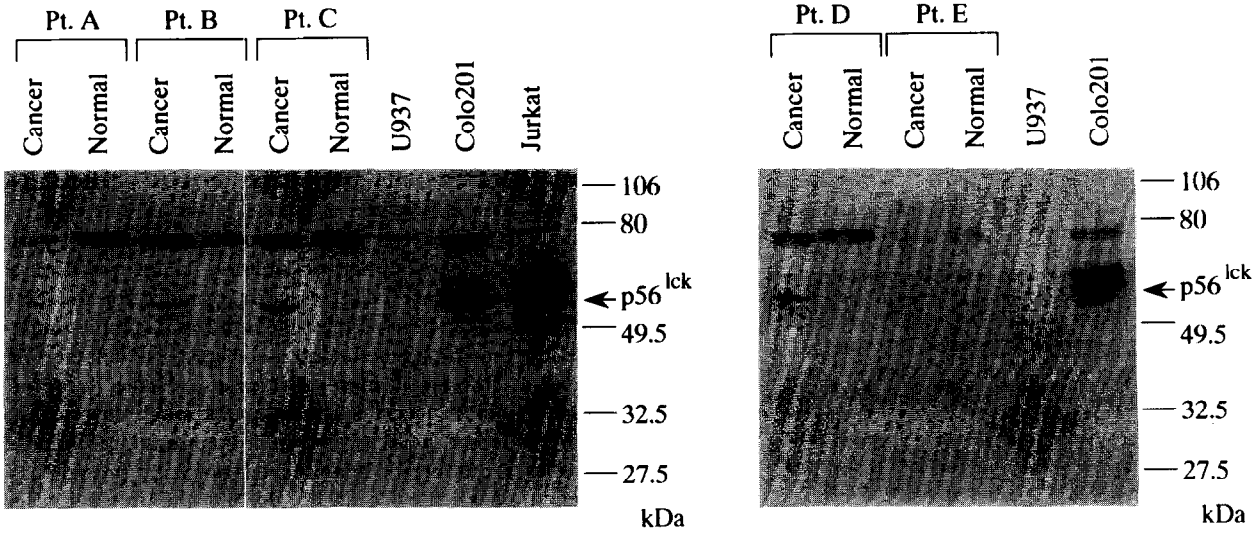

Figure 1. Immunoblot analysis of the lck protein in normal colorectal tissues and in primary colorectal cancers. Arrows indicate p56^{lck}. The position of the molecular weight markers are indicated in kDa on the right. Jurkat and Colo201 are positive controls and U937 is a negative control (see text for details).

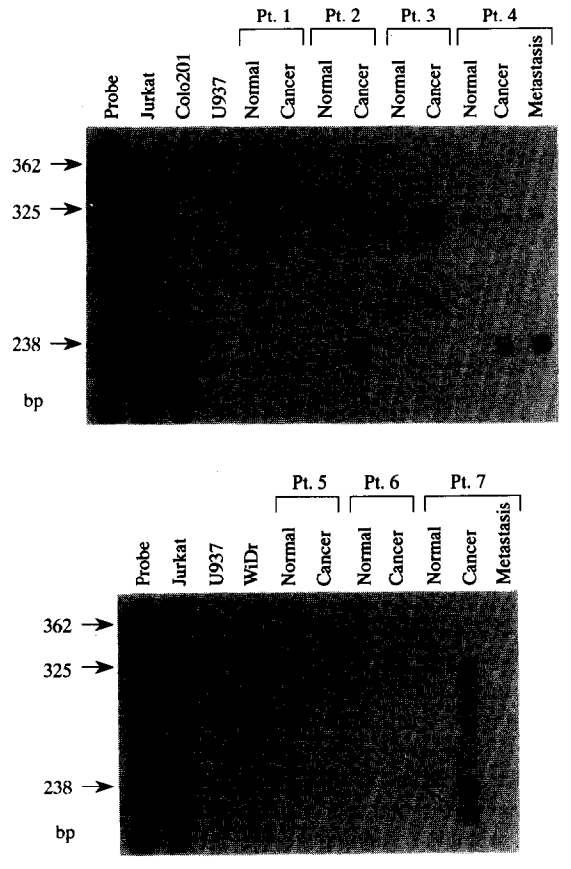

Figure 2. RNase protection assay of *LCK* transcript. 362, 325 and 238 base pair bands correspond to the probe and the *LCK* transcripts from the upstream and the downstream promoter, respectively. Normal, normal colorectal mucosa; Cancer, primary colorectal cancer; Metastasis, hepatic metastasis; bp, base pairs.

the downstream promoter was observed. In patients 1 and 5, small amounts of the *LCK* transcript initiated from the downstream promoter were detected in cancer tissue which was obviously more abundant than that in normal adjacent mucosa. These differences were not due to the failure of the isolation of RNA, because β-actin message was analysed as an internal control, and in all samples used in this study, a similar level of its message was detected (Figure 3 and data not shown). We tested 18 cases of primary colorectal cancer and

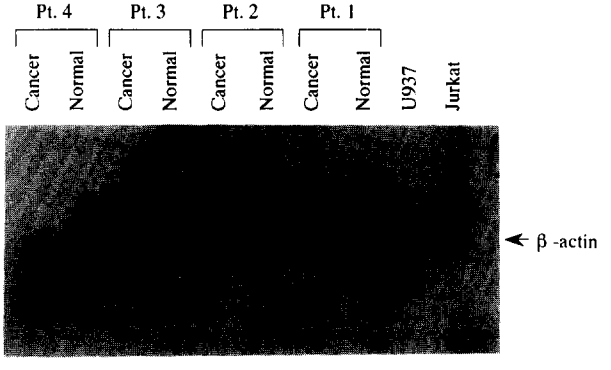

Figure 3. Northern blot analysis of β-actin message in colonic samples. An arrow denotes message of human β-actin. Exposure time: 1 day. Pt, patient; normal, normal colorectal mucosa; Cancer, primary colorectal cancer.

the level of the downstream promoter-directed *LCK* transcript expression in each sample was determined by way of densitometric analysis. Clinical features of each case are listed in Table 1. The values of the *LCK* mRNA level were quantitated based on values for β-actin. The level of *LCK* mRNA from the downstream promoter in normal mucosa from patient 1 was scored as 1 and the relative intensity was calculated based on this in each sample (Figure 4). In patients 11 and 18, we could not detect significant message either in normal mucosa or in cancer, so that the relative intensity was not calculated. The mean values for normal and cancer samples were 0.93 and 8.6, respectively. 3 patients had hepatic colorectal metastases and *LCK* message in metastatic lesions in patients 4 and 7 were also analysed. In patient 4, the level of the downstream promoter-directed *LCK* expression was increased in the metastatic lesion compared with the primary tumour. In patient 7, *LCK* transcript initiated from the downstream promoter was also detected in metastasis, the level of which was greater than that in normal colonic mucosa. Relative intensity of the downstream promoter-initiated message in normal, primary and metastatic lesions in patients 4 and 7 is shown in Figure 5. Normal colorectal tissues and colorectal cancers contained various amounts of the upstream promoter-initiated *LCK* transcript, which is due to the existence of lymphocytes in colonic tissues. Overall, the results indicate that the downstream promoter-initiated *LCK* mRNA was expressed at low level in normal colorectal epithelia and was strongly overexpressed in 3 of 18 colorectal tumours.

DISCUSSION

$p56^{lck}$ is expressed in some human colon carcinoma cell lines, which suggests that lck kinase is associated with colorectal carcinogenesis as are other *SRC*-family protein tyrosine kinases, such as c-src and c-yes. The expression, however, of $p56^{lck}$ in human normal colonic epithelia and colonic cancers remains to be clarified. The presence of lymphocytes which also express $p56^{lck}$ means that lck expressed in colonic epithelia has to be discriminated from that in lymphocytes in order to investigate lck expression in colorectal cancer cells. In this study, utilising the differences of the promoter usage in the transcription of *LCK* between colonic cancer cell lines and peripheral blood lymphocytes, we analysed the expression of the *LCK* message in colorectal normal mucosa and cancer by a RNase protection assay. The downstream promoter-directed *LCK* transcript should be detected if colorectal cancer cells express lck. As shown in Figure 2, normal colorectal mucosae contained traces of the downstream promoter-directed *LCK* message. To confirm the existence of the downstream promoter-initiated *LCK* transcript in colonic tissues, we performed reverse transcription-polymerase chain reaction and detected polymerase chain reaction product for the downstream promoter-derived message in normal colonic mucosae (data not shown). Veillette and associates [21] detected *LCK* mRNA at low levels in normal colonic tissue, but not in human colonic mucosal cell cultures. Although they suggested that detection of the *LCK* mRNA in the normal colonic tissue is due to a number of lymphocytes normally present in the colonic mucosa, they could not exclude the possibility that a small proportion of the colonic epithelium, not represented in the culture of normal colonic mucosal cells, expresses *LCK*. Our demonstration of the downstream promoter-directed *LCK* transcript in normal colonic tissue strongly indicates

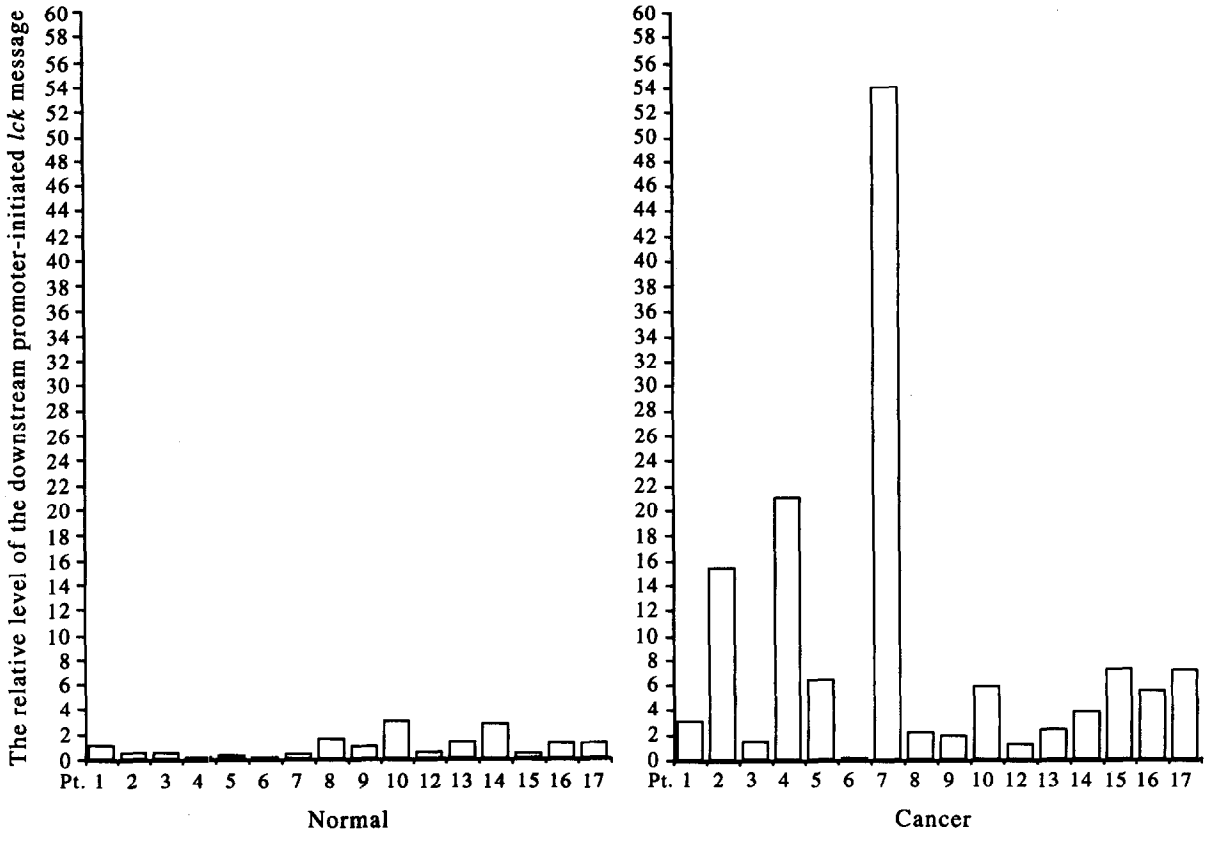

Figure 4. The relative level of the downstream promoter-directed *LCK* transcript in colorectal cancer specimens and normal colorectal tissues. The level of steady state *LCK* message in normal colon sample from patient 1 was scored as 1 and relative intensity was calculated based on this. In patients 11 and 18, detectable message was not observed either in cancer or in normal, so the relative intensity was not calculated.

that low levels of *LCK* message are expressed in colonic epithelial cells.

The expression of the downstream promoter-initiated *LCK* message was augmented in some colorectal cancer samples relative to that in normal mucosa (Figure 2). In 3 of 18 cases (patients 2, 4 and 7), the downstream promoter-initiated *LCK* message was strongly expressed in the cancer specimen and the relative values of *LCK* mRNA level in these tumours were more than 10-fold higher than the average score of normal mucosae, which could be significant enough to play an important role in the development of cancer (Figure 4). In another 7 tumours, the amount of the downstream promoter-initiated message was also larger than that in normal samples, but the relative value was between 3 and 10. Veillette and colleagues [21] reported the expression of *LCK* mRNA in colon cancer cell lines. They analysed 13 cell lines and *LCK* message was detectable in all tested. The level of *LCK* mRNA varied, being very low but detectable in 5 cell lines, intermediate in 5 and high in 3. Together with their findings that lck is expressed in plural colorectal carcinoma cell lines, it is reasonable to conclude that lck is overexpressed and related to tumorigenesis in some colorectal cancer, and the degree of its contribution will vary among tumours.

As normal colon mucosa contains large amounts of stromal cells, it is possible this would dilute out the lck signal expressed in epithelial cells. In comparison, cancer samples might contain a higher ratio of epithelial, that is, cancer cells. However, non-epithelial cells also occur in cancer samples, so that the difference in the percentage of epithelial cells alone could not explain such drastic *LCK* overexpression seen in patients 2, 4 and 7.

Although resting peripheral lymphocytes contain only the upstream product of the *LCK* gene, it is possible that activated lymphocytes contain both products. To exclude this possibility, we investigated promoter usage during *LCK* expression in peripheral lymphocytes activated by phorbol myristate acetate and calcium ionophore. Consistent with previous reports [23, 30], we observed a transient downregulation of the upstream promoter-directed *LCK* expression, but never detected downstream promoter-directed *LCK* transcript in activated lymphocytes (data not shown).

2 patients with the strongest *LCK* overexpressing colorectal cancers had hepatic and lymph node metastases (patients 4 and 7, Table 1), which may suggest that augmentation of lck kinase could be associated with the degree of biological malignancy of the tumour. We are now increasing the number of samples for more precise evaluation of the clinical relevance of lck expression in colorectal cancer. In both patients 4 and 7, the downstream promoter-initiated *LCK* message was expressed in hepatic metastases as well as in primary cancers, which confirms the expression of lck in colorectal cancer cells.

Contrary to our results, Park and coworkers [16] reported that they did not detect lck activity in colon tumours. Even if lck is not expressed in colonic cancer cells, large numbers of lymphocytes infiltrate into the tumour, so that lck kinase activity should be immunoprecipitated from surgically resected cancer specimens. As shown in Figure 2, we also detected the upstream promoter-initiated *LCK* message in

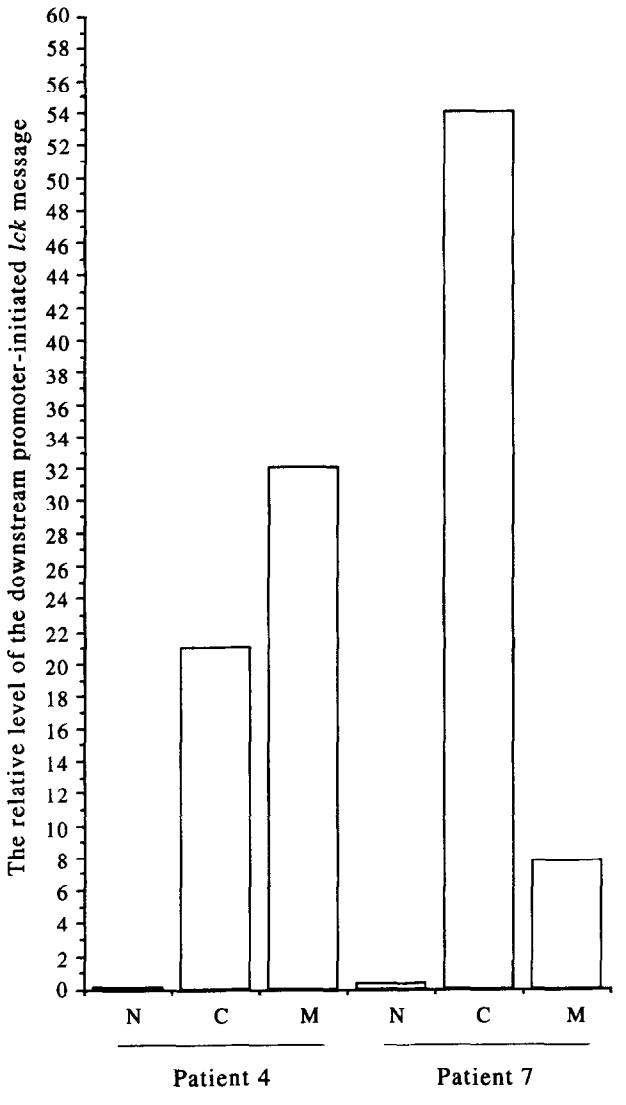

Figure 5. The relative intensity of the downstream promoter-directed *LCK* transcript in normal colorectal mucosa, primary colorectal cancer and hepatic metastasis. The level of steady state *LCK* message in normal colon sample from patient 1 was scored as 1 and relative intensity was calculated based on this. N, normal colorectal mucosa; C, primary colorectal cancer; M, hepatic metastasis.

normal colonic tissues and in colon cancer specimens supporting the fact that many lymphocytes were contained in such samples. Thus, the failure to detect lck activity in colonic tissues by Park and colleagues [16] is probably because the sensitivity of their immune complex and kinase assay was lower than that of our immunoblot and RNase protection assay.

In this report, we have demonstrated that *LCK* is expressed from the downstream promoter at low level in normal colorectal mucosae. In addition, the expression of the downstream promoter-directed *LCK* mRNA is augmented in some colonic cancers relative to that in normal adjacent colonic mucosae. Our results indicate that augmentation of the lck kinase may be involved in the development of some colorectal cancers.

1. Fearon ER, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990, **61**, 759–767.
2. Nigro JM, Baker SJ, Preisinger AC, *et al.* Mutations in the *p53* gene occur in diverse human tumour types. *Nature* 1989, **342**, 705–708.
3. Bos JL, Fearon ER, Hamilton SR, *et al.* Prevalence of *ras* gene mutations in human colorectal cancers. *Nature* 1987, **327**, 293–297.
4. Hunter T, Cooper JA. Protein-tyrosine kinases. *Ann Rev Biochem* 1985, **54**, 897–930.
5. Rudd CE. CD4, CD8 and the TCR–CD3 complex: a novel class of protein-tyrosine kinase receptor. *Immunol Today* 1990, **11**, 400–406.
6. Sefton BM. The *lck* tyrosine protein kinase. *Oncogene* 1991, **6**, 683–686.
7. Amrein KE, Sefton BM. Mutation of a site of tyrosine phosphorylation in the lymphocyte-specific tyrosine protein kinase, $p56^{lck}$, reveals its oncogenic potential in fibroblasts. *Proc Natl Acad Sci USA* 1988, **85**, 4247–4251.
8. Bishop JM. Viral oncogenes. *Cell* 1985, **42**, 23–38.
9. Cartwright CA, Eckhart W, Simon S, Kaplan PL. Cell transformation by $pp60^{c\text{-}src}$ mutated in the carboxy-terminal regulatory domain. *Cell* 1987, **49**, 83–91.
10. Kmiecik TE, Shalloway D. Activation and suppression of $pp60^{c\text{-}src}$ transforming ability by mutation of its primary sites of tyrosine phosphorylation. *Cell* 1987, **49**, 65–73.
11. Marth JD, Cooper JA, King CS, *et al.* Neoplastic transformation induced by an activated lymphocyte-specific protein tyrosine kinase ($pp56^{lck}$). *Mol Cell Biol* 1988, **8**, 540–550.
12. Bolen JB, Veillette A, Schwartz AM, DeSeau V, Rosen N. Activation of $pp60^{c\text{-}src}$ protein kinase activity in human colon carcinoma. *Proc Natl Acad Sci USA* 1987, **84**, 2251–2255.
13. Bolen JB, Veillette A, Schwartz AM, DeSeau V, Rosen N. Analysis of $pp60^{c\text{-}src}$ in human colon carcinoma and normal human colon mucosal cells. *Oncogene Res* 1987, **1**, 149–168.
14. Cartwright CA, Kamps MP, Meisler AI, Pipas JM, Eckhart W. $pp60^{c\text{-}src}$ activation in human colon carcinoma. *J Clin Invest* 1989, **83**, 2025–2033.
15. DeSeau V, Rosen N, Bolen JB. Analysis of $pp60^{c\text{-}src}$ tyrosine kinase activity and phosphotyrosyl phosphatase activity in human colon carcinoma and normal human colon mucosal cells. *J Cell Biochem* 1987, **35**, 113–128.
16. Park J, Meisler AI, Cartwright CA. c-Yes tyrosine kinase activity in human colon carcinoma. *Oncogene* 1993, **8**, 2627–2635.
17. Garcia R, Parikh NU, Saya H, Gallick GE. Effect of herbimycin A on growth and $pp60^{c\text{-}src}$ activity in human colon tumor cell lines. *Oncogene* 1991, **6**, 1983–1989.
18. Talamonti MS, Roh MS, Curley SA, Gallick GE. Increase in activity and level of $pp60^{c\text{-}src}$ in progressive stages of human colorectal cancer. *J Clin Invest* 1993, **91**, 53–60.
19. Termuhlen PM, Curley SA, Talamonti MS, Saboorian MH, Gallick GE. Site-specific differences in $pp60^{c\text{-}src}$ activity in human colorectal metastases. *J Surg Res* 1993, **54**, 293–298.
20. Marth JD, Peet R, Krebs EG, Perlmutter RM. A lymphocyte-specific protein-tyrosine kinase gene is rearranged and overexpressed in the murine T cell lymphoma LSTRA. *Cell* 1985, **43**, 393–404.
21. Veillette A, Foss FM, Sausville EA, Bolen JB, Rosen N. Expression of the *lck* tyrosine kinase gene in human colon carcinoma and other non-lymphoid human tumor cell lines. *Oncogene Res* 1987, **1**, 357–374.
22. Takadera T, Leung S, Gernone A, *et al.* Structure of the two promoters of the human *lck* gene: differential accumulation of two classes of *lck* transcripts in T cells. *Mol Cell Biol* 1989, **9**, 2173–2180.
23. Nakamura K, Koga Y, Yoshida H, Kimura G, Nomoto K. Differential expression of two *lck* transcripts directed from the distinct promoters in HTLV-I^+ and HTLV-I^- T-cells. *Int J Cancer* 1991, **48**, 789–793.
24. Sartor O, Gregory FS, Templeton NS, Pawar S, Perlmutter RM, Rosen N. Selective expression of alternative *lck* mRNAs in human malignant cell lines. *Mol Cell Biol* 1989, **9**, 2983–2988.
25. Moroi Y, Koga Y, Nakamura K, Ohtsu M, Kimura G, Nomoto K. Accumulation of $p60^{lck}$ in HTLV-I-transformed T cell lines detected by an anti-Lck monoclonal antibody, MOL171. *Jpn J Cancer Res* 1991, **82**, 909–915.
26. Salcedo TW, Kurosaki T, Kanakaraj P, Ravetch JV, Perussia B. Physical and functional association of $p56^{lck}$ with FcγRIIIA (CD16) in natural killer cells. *J Exp Med* 1993, **177**, 1475–1480.

27. Chomczynski P. A reagent for the single-step simultaneous isolation of RNA, DNA and proteins from cell and tissue samples. *BioTechniques* 1993, **15**, 532–536.
28. Koga Y, Caccia N, Toyonaga B, *et al.* A human T cell-specific cDNA clone (YT16) encodes a protein with extensive homology to a family of protein-tyrosine kinases. *Eur J Immunol* 1986, **16**, 1643–1646.
29. Marth JD, Lewis DB, Cooke MP, *et al.* Lymphocyte activation provokes modification of a lymphocyte-specific protein tyrosine kinase (pp56^{lck}). *J Immunol* 1989, **142**, 2430–2437.
30. Marth JD, Lewis DB, Wilson CB, *et al.* Regulation of pp56^{lck} during T-cell activation: functional implications for the *src*-like protein tyrosine kinases. *EMBO J* 1987, **6**, 2727–2734.

Acknowledgement—We thank Dr Kabemura for providing us with colorectal cancer specimens.