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NO CORRELATION BETWEEN L-*myc* RESTRICTION FRAGMENT LENGTH POLYMORPHISM AND MALIGNANCY OF HUMAN COLORECTAL CANCERS

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The correlations of the restriction fragment length polymorphism (RFLP) pattern of L-*myc* with the progressive state of cancer and metastases to lymph nodes or other organs were examined in 35 cases of human colorectal cancer by χ^2 analysis. No significant correlation was found.

Key words: L-*myc* — Restriction fragment length polymorphism — Colorectal cancer

Oncogene research has provided much information on the biology of tumors. It is crucially important, however, to study whether this information is applicable to clinical diagnoses or prognostic factors. Amplification and expression of N-*myc* have been reported to be correlated with the stage and overall survival of patients with neuroblastoma.[1-3] Neuroblastomas with a more favorable prognosis and in an earlier stage at the time of diagnosis were reported to have higher amounts of the Ha-*ras* product.[4] Furthermore, HER-2/*neu* amplification was found to be a significant predictor of both overall survival and the relapsing time of patients with breast cancer.[5]

Studies using RFLP have been made on whether genetically predisposing properties affect the incidence or prognosis of certain types of cancer.[6-10] Recently, Kawashima *et al.* proposed that the pattern of L-*myc* RFLP, detected as 10 kb (L) and 6.6 kb (S) bands of *Eco*RI digests of DNA,[11] correlated with the metastatic property of lung cancer; namely, L-homozygous patients had few lymph node metastases, whereas L-S heterozygous or S-homozygous patients, whose DNA gave an S-band, almost always had metastases. Furthermore, they found that one L-*myc* RFLP, the L-L type, an indicator of low progressiveness of tumors, is predominant in patients with colon cancer.[12] In order to obtain more reliable information, the correlations of oncogenes and clinical features must be studied in more cases. In this work, we studied the L-*myc* RFLP in 35 cases of colorectal cancer in relation to the progression of tumors and the presence of metastases.

Samples of 35 colorectal cancers and 31 corresponding regions of normal mucosa from the same patients were obtained from 21 males of 29 to 82 years old and 14 females of 35 to 85 years old. The samples were provided by the Pathology Division, National Cancer Center Research Institute, Tokyo, or the First Department of Surgery, Nippon Medical School Hospital, Tokyo. These resected tissues were promptly frozen in liquid nitrogen and stored at $-80°$.

High-molecular-weight DNA was isolated from tissues by the method of Perucho *et al.*[13] Samples of 10 μg of DNA were digested with *Eco*RI, subjected to electrophoresis in 0.7% agarose gel, and transferred to a nitrocellulose filter as described by Southern.[14] Hybridization was carried out under moderately stringent conditions (at 42°, 50% formamide, 0.65 *M* NaCl). For detection of L-*myc* RFLP, an *Sma*I-*Eco*RI 1.8 kb fragment was generated from pJB327,[15] provided by the Japanese Cancer Research Resources Bank, Tokyo. The fragment was labeled with ^{32}P-dCTP by nick translation and had a specific radioactivity of more than 2×10^9 cpm/μg DNA.

The results of Southern blot analysis and profiles of the tumors are summarized in Table I. Of the 35 cancers, 34 were adeno-

Table I. Distribution of L-*myc* RFLP in 35 Human Colorectal Cancers

	L-*myc* RFLP pattern			Total
	L-L	L-S	S-S	35
	7	21	7	
Histopathology				
adenocarcinoma	6	21	7	34
squamous cell carcinoma	1	0	0	1
Histopathological stage[a]				
I	0	3	0	3
II	2	4	4	10
III	1	5	2	8
IV	0	1	0	1
V	4	8	1	13
Dukes' classification				
A	0	3	0	3
B	2	5	4	11
C	5	13	3	21
Lymph node metastasis				
−	2	8	4	14
+	5	13	3	21
Metastasis to other organs[b]				
−	4	13	6	23
+	3	8	1	12

a) The histopathological stage was evaluated according to General Rules for Clinical and Pathological Studies on Cancer of Colon, Rectum and Anus (1986).[16]
b) Metastasis to the liver, ovary or urinary bladder. There was no metastasis to the brain or lung.

carcinomas and one was a squamous cell carcinoma. The distribution of the colorectal cancers according to the criteria of the histopathological stages and Dukes' classifications are shown in Table I. In 21 cases, lymph node metastases were detected at the time of operation. Metastases to other organs were detected in 12 cases. The correlations between the L-*myc* RFLP pattern and progression and metastasis in these cases were studied.

Of these cases, 7 were the L-L type, 21 were the L-S type and 7 were the S-S type. This distribution is compatible with that found by Nau *et al.* in 83 samples, including cancer tissues, cancer cell lines and tissues of normal individuals.[15] Based on a study of 10 cases, Kawashima *et al.* reported that most Japanese patients with colon cancer have the L-L type.[12] But, our data suggest that there is no remarkable deviation in the L-*myc* RFLP pattern in Japanese patients with colorectal cancer. It has also been reported that there is no loss of L-*myc* heterozygosity in lung cancer.[11] We found that this was also true in the cases of colorectal cancer; the pattern of L-*myc* RFLP and the intensities of the two fragments were the same in the DNAs from the samples of colorectal cancer and of normal tissue from the same patients.

Three of the L-L type patients had cancers in stage I, II or III, and 4 had cancers in stage IV or V. On the other hand, 18 of the L-S and S-S type patients had cancers in stage I, II or III, and 10 had cancers in stage IV or V. Thus there was also no significant difference between the L-L and L-S plus S-S types in the stages of progression of the cancers ($P>0.2$).

Of the L-L type patients, 5 had lymph node metastases and 2 did not. Of the L-S plus S-S type patients, 16 had lymph node metastases and 12 did not. Moreover, 3 of the L-L type patients had metastases to other organs and 4 did not, while 9 of the L-S and S-S type patients had metastases to other organs and 19 did not. Thus there was also no significant difference between the L-L homozygous and the L-S heterozygous or S-S homozygous groups in the proportions of patients with metastases to either lymph nodes or other organs (both $P>0.4$).

In this study, we examined the relationship between the L-*myc* RFLP pattern and the clinical features of colorectal cancer in 35 cases. The discrepancy between our conclusion and that of others may be because we examined more cases. No clear marker is yet available for the early diagnosis or for the prognosis of colorectal cancer. Such a marker is urgently required.

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