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Short communication

Restriction fragment length polymorphism of the L-*myc* gene is not a prognostic factor in bladder cancer patients

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Summary The L-*myc* restriction fragment length polymorphism has been suggested to be of prognostic significance in some types of primary tumours. We examined the prognostic and susceptibility significance of the L-*myc* genotype in a group of 98 bladder cancer patients. The L-*myc* genotype did not correlate with any pathologic parameter and does not offer any clinical utility in patients with bladder cancer.

Keywords: proto-oncogenes; restriction fragment length polymorphism; bladder cancer; prognostic factors

The restriction enzyme Eco RI identifies a two-allele system for the L-*myc* gene with fragment sizes of 10.0 and 6.6 kilobases and results in three possible allelotypes: homozygotic for the large allele (LL), homozygotic for the small allele (SS) or heterozygotic (LS). The prognostic significance of the L-*myc* allelotype of genomic DNA has been studied previously in several tumours (lung, colorectal, breast, soft tissue, leukaemia, lymphoma, stomach, kidney, oral cavity and liver). However, conficting results were reported. In this study we have investigated the L-*myc* genotype of 98 primary bladder cancer patients in an attempt to determine its utility as a prognostic, and as a susceptibility, factor in a Catalonian population.

MATERIALS AND METHODS

Ninety-eight patients (88 males, 10 females, aged 38–91), diagnosed at Fundació Puigvert as having primary bladder cancer, were studied for L-*myc* restriction fragment length polymorphism (RFL); P 53 were superficial bladder tumours and 45 were invasive tumours. In addition, 110 normal DNA specimens from healthy blood donors (66 males, 44 females, aged 2–81) were analysed to investigate the genotype frequency of L-*myc* proto-oncogene.

High molecular weight DNA was isolated by standard phenol–chloroform methods. Restriction digests of DNA were performed with endonuclease Eco RI under standard conditions, electrophoresed in a 0.8% agarose gel, denaturated, transferred to Hybond® nylon membrane (Amersham) and hybridized with a ^{32}P-labelled probe by the method essentially described by Southern (1975).

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All results obtained in these studies were analysed for statistical significance by the χ^2 test with Yates' correction for adjustment when necessary. Differences between the two populations were judged significant at $P < 0.05$. Kaplan–Meier curves were constructed to analyse patient survival between different genotypes (log-rank test).

RESULTS

Eco RI-digested DNA probed with the L-*myc* probe results in two fragments of 10 kb (L) and 6.6 kb (S), which are due to an Eco RI restriction site polymorphism (Figure 1). The distribution of the three genotypes (LL, LS, SS) in the control and patient groups is shown in Table 1. Chi-squared analysis showed that there was no difference between the distribution of the three genotypes of patient group and controls, and all are in accordance with Hardy–Weinberg equilibrium.

When the patients in the current study were examined for a relationship between the presence of S-alleles (LS or SS individuals) and distant metastasis, no association was found ($P = 0.51$). Nor was there a significant association between homozygosity for the

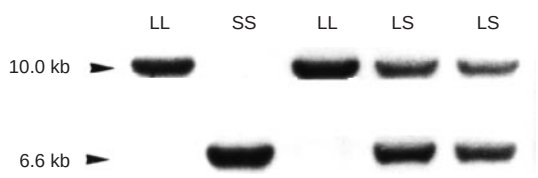

Figure 1 Examples of Southern hybridization analysis of the L-*myc* gene. DNA samples obtained from peripheral leucocytes of five control individuals are shown. Genotypes of lanes 1 and 3 were LL, that of lane 2 was LS, and those of lanes 4 and 5 were SS

Table 1 Distribution of L-*myc* genotypes and allele frequencies in patients and normal controls

	Genotypes						Allele frequency		
	LL	LS	SS	Total	χ^2	(*P*)	L	S	(*P*)
Controls	40	45	25	110			0.568	0.432	
Patients	32	45	21	98	0.546	> 0.05	0.556	0.444	> 0.05

Table 2 Data on patients with bladder cancer and the distribution of the L-*myc* genotypes

		DNA pattern of L-*myc* no. of cases		
Total number	**Total**	**LL**	**LS**	**SS**
Age (years)				
< 60	10	3	6	1
61–70	37	16	16	5
> 70	51	14	26	11
Sex				
Male	88	28	44	16
Female	10	4	1	5
Smoking				
Yes	71	23	34	14
No	27	9	11	7
Grade of differentiation				
G1 + G2	45	14	19	12
G3 + anaplas. + indifferentiated	53	18	26	9
TNM stages				
Ta + T1	53	18	25	10
T2 + T3 + T4	45	14	20	11
Recurrence[a]				
R +	46	11	26	9
R –	52	21	19	12
Distant metastasis[b]				
M +	28	7	15	6
M –	68	25	29	14
Nodal metastasis[c]				
N +	15	4	9	2
N –	71	26	30	15
Urethral involvement				
U +	5	–	3	2
U –	93	32	42	19
Prostate involvement[d]				
P +	9	2	5	2
P –	79	26	39	14

[a]Number of patients with previous relapses (R+) versus number of patients without previous relapses (R–). [b]This variable was not possible to examine in two cases. [c]This variable was not possible to examine in 12 cases. [d]Ten female patients were excluded of this examination.

S-allele and nodal metastasis ($P = 0.46$). Further analysis revealed no significant associations with grade of differentiation of tumours, TNM stages, number of previous and subsequent relapses, and urethral and prostate involvement (Table 2). No association was observed between L-*myc* RFLP and survival of bladder cancer patients (Figure 2).

DISCUSSION

An Eco RI polymorphism of the L-*myc* proto-oncogene was reported with the initial description of the gene (Nau et al, 1985) and the site of the polymorphism subsequently located in the second intron (Kaye et al, 1988). Studies of this polymorphism have linked it to both cancer susceptibility and prognosis. The role of L-*myc* in cancer susceptibility has been implied by studies showing differences in genotype frequencies between cancer patients and controls (Kato et al, 1990; Dolcetti et al, 1991; Crossen et al, 1994). The role of L-*myc* in prognosis has been suggested by studies showing that, among cancer patients, those that carry an S-allele (either LS or SS genotype) have earlier lymph node involvement or metastasis, or poorer survival than those who have genotype LL (Kakehi and Yoshida, 1989; Champeme et al, 1992; Kawashima et al, 1992). In contrast, Taylor et al (1993) reported a protective effect for the SS genotype in hepatocellular carcinoma. Hence, the association of the L-*myc* genotype with susceptibility and prognosis appears to vary with tumour type.

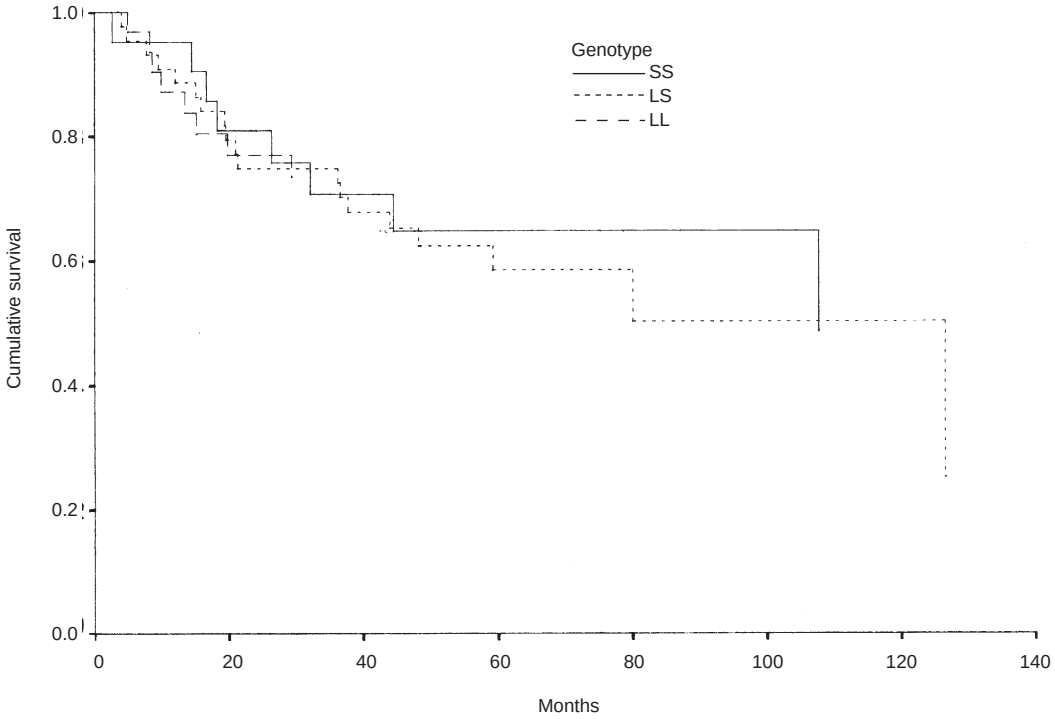

Figure 2 Survival curves of bladder cancer patients with LL, LS and SS L-*myc* RFLP (log-rank test, $P = 0.53$)

In this study, we examined the relationship between the L-*myc* RFLP pattern and the clinical features of bladder cancer in 98 cases. A preponderance of the LL, LS or SS fragments was not observed in the bladder cancer patients compared to normal healthy individuals; thus, presence of a particular allele did not indicate predisposition to bladder cancer. Similar L-*myc* allelic frequencies were also found in previously reported analyses.

Our results fail to support the hypothesis that the L-*myc* locus is involved in a genetic predisposition to bladder cancer. These results confirm those of Ikeda et al in colorectal cancer (1988), Tefre et al in lung cancer in Norway (1990), Ishizaki et al (1990) and Champeme et al (1992) in breast cancer, Saranath et al (1990) in oral cancer, Weston et al (1992) in lung cancer in the USA, Mironov et al (1994) in gastric cancer in Russia and Presti et al (1996) in renal cancer. On the other hand L-*myc* RFLP is correlated with the extent of metastasis of lung cancer in Japan (Kawashima et al, 1988, 1992) and in renal cancer (Kakehi and Yoshida, 1989); Ishizaki et al (1990) also reported that the presence of the S-allele is associated with poor prognosis due to metastasic lesion in gastric cancer. Kato et al (1990) proposed that Japanese men with the S-allele may be prone to development of osteosarcoma and Saranath et al (1990) observed that patients with oral cancer with a genotype including an S fragment are more likely to develop a poorly to moderately differentiated tumour, or a larger tumour, than patients without an S fragment.

Our results show that the L-*myc* genotype does not correlate with tumour grade or stage in patients with bladder carcinoma. No significant correlation was observed between the L-*myc* genotype and the presence of nodal metastases or distant metastases at the time of surgery or with subsequent disease relapse.

This study did not demonstrate an association between the L-*myc* genotype and disease status in bladder cancer. It seems that L-*myc* polymorphism is not suitable as a prognostic and susceptibility marker in the bladder cancer patients studied.

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