

Increased Frequency of the S-allele of the L-myc Oncogene in Breast Cancer

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Abstract

Background: Association between restriction fragment length polymorphisms (RFLP) of known oncogenes and a predisposition to develop cancer have been postulated. The L-myc gene is a potential molecular marker associated with cancer susceptibility as well as metastasis, prognosis, and adverse survival. Our aim was to test the hypothesis that there was an association between L-myc S allele in breast cancer and a predisposition to the disease.

Materials and Methods: The distribution of L-myc polymorphism in 56 patients with breast cancer was determined by polymerase chain reaction-based restriction fragment length polymorphism and compared with that of 51 healthy control subjects.

Results: The allele frequencies of L and S in breast cancer patients were 0.70 and 0.30, respectively and those in normal individuals were 0.54 and 0.46, respectively. This difference was primarily the result of a high frequency of the S allele among breast cancer patients compared to controls. The frequency of S allele was significantly higher in breast cancer patients than in normal individuals ($p < 0.01$). No correlation was observed between the presence of L-myc S allele and several parameters of each patient's history or characteristics of tumor.

Conclusion: Our results suggested that L-myc polymorphism may be significant in an individual's susceptibility to breast cancer in Turkey and may be useful for identifying patients at high risk of developing breast cancer.

Introduction

Breast cancer is a genetic disease, with most breast cancer cases resulting from a dysregulation of genetically determined cellular pathways. The occurrence of cytogenetic abnormalities affecting chromosome 1 in a number of breast tumors led us to examine, at the molecular level, loci located on chromosome 1 in primary breast tumor DNAs (1). Association between restriction fragment length polymorphism (RFLPs) of known oncogenes and a predisposition to develop cancer have been reported by a number of authors (2,3). Since the cloning of L-myc gene in 1985, an enormous amount of research has been conducted to help elucidate the importance of this gene in human malignancy. Eco RI restricted human DNAs show fragment length polymorphism of L-myc (chromosome 1p 32) defined by 2 alleles: 10.0 kb (L) and 6.6 kb (S) fragments (4). Previous studies have reported that patients showing either the homozygous S-band (S-S) or the heterozygous S and L-band (L-S) suffered a much higher incidence of metastasis and

a poor prognosis for several cancers (5–8). Other investigators, however, failed to find such an association in lung cancers (9–12), colorectal cancers (13), non-Hodgkin's lymphomas (14), and acute lymphocytic leukemias (15).

In the present study PCR-RFLP method was used to analyze the distribution of L-myc polymorphism both in patients with breast cancer and healthy Turkish females in an attempt to resolve the debate as to whether any relationship exists between polymorphism and the risk of developing breast cancer.

Materials and Methods

Subjects

L-myc gene polymorphism has been studied among 56 breast cancer patients (mean age 56.9 ± 10.73 ; age range 32–74 years) and 51 age-matched female non-malignant healthy subjects. Healthy persons without any malignancy were selected for the control group (mean age; 54.3 ± 12.01 ; age range 32–74 years). Subjects were selected from two education hospitals in Istanbul between 2000–2001. Diagnosis of breast cancer done by surgical clinics relied upon mammography, ultrasonography, and finally pathological examination. Controls were selected from

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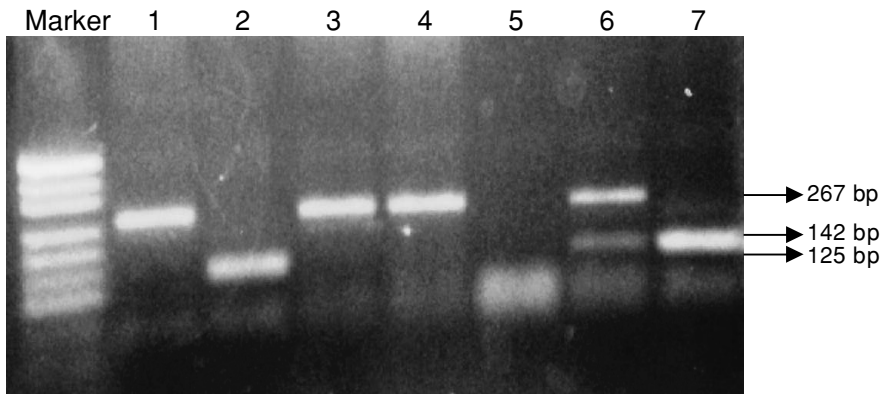


Fig. 1. Direct visualization of PCR products by ethidium bromide staining. A 267-base pair L-myc fragment was amplified, cleaved with Eco RI, and electrophoresed on a 2% agarose gel. Results from seven representative breast cancer patients are shown. Lane 1: LL homozygote; lane 2: SS homozygote; lane 3-4: LL homozygote; lane 5: no PCR product; lane 6: LS heterozygote; lane 7: SS homozygote. The marker band uses (bp) are from top to bottom: 501, 404, 331, 242, 190, 147, 111, and 67.

surgery polyclinics and clinics among the people who had no proven malignant disease or disease history and were in the same age group with the breast cancer patients. All the breast cancer patients and controls were citizens of the Turkish republic; no minority people were included in this study. Detailed medical history, physical examination, and pathological diagnosis were performed for all patients in the study. The samples were collected before any chemotherapeutic or radiation therapy treatment had been started. A standardized questionnaire was administrated to collect data concerning age, sex, family history of breast cancer, and family history of any kind of cancer for only 56 breast patients from whom we obtained blood samples.

Isolation of DNA

Blood specimens from all subjects were collected into tubes containing EDTA. DNA was isolated from the blood leukocytes in 10 ml EDTA by the method of Miller et al. based on sodium dodecyl sulphate lysis, ammonium acetate extraction, and ethanol precipitation (16).

Polymerase Chain Reaction (PCR) for L-myc Oncogene

Template DNA (0.5–1.0 ug) was used in a PCR under sterile conditions. 100 ng of primer was used for the reaction. The forward primer was 5'-AGT TCA CTC ACA GGC CAC AT-3' and the reverse primer was 5' TGC ATA TCA GGA AGC TTG AG-3' in a volume of 50 μ l containing 3 mM MgCl₂, 50 mM KCl, 10 mM Tris-HCl (pH:8.4), 0.5 mM of each dNTP (MBI Fermentas), and 1 unit of Taq polymerase (MBI Fermentas). Amplification was carried out in a DNA thermal Cycler (MJ Research Techne) for 30 cycles with denaturation steps at 94°C for 30 seconds, annealing at 50°C for 1 min, and extension at 74°C for 1 min. The PCR product exhibited a 267 base pair fragment. The amplified DNAs were all digested with 5 units Eco RI (MBI Fermentas) at 37°C for 1 h. The digested DNA fragments were separated by gel electrophoresis on 2% agarose gel in 1xTris borate EDTA buffer and DNA visualized by ethidium bromide staining. Oncogene polymorphism was typed by visualization under ultraviolet

light and was photographed with a Polaroid camera. The responsible L-myc RFLP alleles were identified in each sample (9). The three genotypes were the L-L homozygote appearing as 267-base pair (bp) fragment, the L-S heterozygote with 267, 142, and 125-bp fragments, and the S-S homozygote with 142 and 125-base pair fragments (Fig. 1).

Statistical Analysis

Statistical analyses were performed using the SPSS version 7.5 including the Chi Square (χ^2) test and allele frequencies composition. L-myc allele frequencies were estimated by gene counting methods. Odds ratios (ODs) and 95% confidence intervals (95% CI) were calculated.

Results

The polymorphic L-myc gene locus was analysed by PCR-RFLP for 56 breast cancer patients and 51 healthy individuals. The L-myc oncogene genotypes and allele frequencies for breast cancer and control subjects are shown in Table 1.

Table 1. Distribution of L-myc restriction fragment length polymorphism genotypes in patients with breast cancer and control subjects

	Control Subjects (n = 51)	Breast Cancer Patients (n = 56)
L-myc genotype		
L-L	0.45 (23)	0.20 (11)
L-S	0.51 (26)	0.69 (39)
S-S	0.04 (2)	0.11 (6)
LL	0.45 (23)	0.20 (11)
LS + SS	0.55 (28)	0.80 (45)
	$\chi^2 = 7.98;$	
	df = 1	
	p = 0.005	

* The number of individuals is shown in parenthesis.

Frequencies of L-L, L-S, and S-S genotypes among the patients with breast cancer were 0.20 (n = 11), 0.69 (n = 39), and 0.11 (n = 6); among the control subjects, there were 0.45 (n = 23), 0.51 (n = 26), 0.04 (n = 2), respectively. There was a significant increase in the frequency of genotypes containing the S allele in the breast cancer population compared with the frequency in the normal population ($\chi^2 = 7.98$; $p = 0.005$) (Table 1.). The relative risk (Odds ratio) of breast cancer for those with the LS plus SS genotypes compared with the LL genotype was 1.91 (95% CI = 1.134–3.200).

The family histories of cancer were analysed in 24 of the patients. Breast cancer patients whose first, second, or third degree relatives have any kind of history of cancer and who have S allele have almost a 1.05 times higher predisposition to breast cancer than those with LL genotype (95% CI = 1.053–7.453).

Discussion

The L-myc gene was initially identified by Nau et al. (4) as a gene with structural similarity to c-myc and N-myc from a human small cell lung cancer cell line and was further characterized and sequenced by Kaye et al. (17). An Eco RI restriction fragment length polymorphism in the L-myc gene can be used to distinguish alleles (4). Many reports have suggested that individuals bearing the “short” (S) allele either have an increased incidence of certain tumors and/or that such tumors manifest a more aggressive behaviour. The associated tumors include soft-tissue sarcomas (18), oral cancers (19), colorectal cancers (6), non-Hodgkin’s lymphoma (20), and breast cancer (21).

Bieche et al. (1) found that a significantly shorter period after relapse was observed for patients with loss of heterozygosity at L-myc in primary tumor DNAs compared with patients with tumor DNAs lacking this alteration. Champeme et al. (21) reported that there was a statistical correlation between L-myc RFLP and lung metastasis in breast cancer patients who relapsed. In this study a significant difference was found in the distribution of L-myc genotypes between breast cancer patients and healthy individuals. Our results supported the hypothesis that the L-myc locus is involved in a genetic predisposition to breast cancer. In contrast to our results, Champeme et al. reported that no differences in the patterns of L-myc RFLP were found between breast cancer patients and healthy individuals (21). The results of our study supported that the L-myc gene is related to genetic susceptibility to breast cancer as Togo et al. (22) suggest. We studied women as a control group for L-myc RFLP analysis. They reported that the statistically significant effect observed in their study was due to the increased prevalence of the S allele in breast cancer group and they analyzed women separately (22).

Multiple epidemiologic studies have documented that a reported history of breast cancer among

relatives is a reproducible predictor of breast cancer risk (In general a “positive family history” of breast cancer confers a relative risk of 2.0 to 3.0 for breast cancer (23). The relationship between risk factors for breast cancer in women with or without a positive family history of this disease has been explored (24, 25). In our study we found that breast cancer patients whose first degree relatives have any kind of history of cancer and who have S allele have almost a 1.05 times higher predisposition to breast cancer than those with LL genotype. Additional work is needed to further characterize the molecular reasons for the increased risk seen in individuals with a positive family history. It would be interesting to study whether or not a specific distribution of the L-myc RFLP can also be observed in breast cancer and to test linkage in high-risk families.

There is no published evidence for the functional significance of polymorphism in second intron of L-myc oncogene. The nucleotide sequence of the S allele has been determined (8) and, as expected, differs by 1 bp in the Eco R1 site. In addition, there was a deletion of 8 bp in intron 2 and it was suggested that these differences might influence the transcription or splicing of the S allele (20). An alternative explanation is that the L-myc gene is not involved but is in linkage disequilibrium with a gene or genes that are important in breast cancer as well as other form of cancer. Linkage disequilibrium of L-myc with another disease-related polymorphic gene(s) appears to be a plausible explanation for the effects observed by various investigators. Spinola et al. report that the nucleotide sequence of the coding and non-coding regions of the L-myc gene (approximately 6000 bp) were analyzed in Italian lung cancer patients. These researchers found no polymorphism in the coding regions, but confirmed the Eco R1 polymorphism (position 3109 of the gene) and determined two additional single nucleotide polymorphism (SNPs) in the 3'-untranslated region (UTR), located at position 5453 and 6130 respectively. They suggested that the differences in allele frequency and linkage disequilibrium patterns in different populations may explain the contrasting results in the Asian and Caucasian population on the role of L-myc EcoR1 polymorphism in lung tumor prognosis (26). According to Mendoza et al., if the L-myc RFLP has linkage disequilibrium with inactivation of a certain tumor suppressor gene (TSG), then the S allele should be correlated with allelic loss around the TSG locus and higher malign potential. But they failed to find an association between the L-myc S allele and LOH around this locus and not support the hypothesis of inactivation of some TSG in linkage disequilibrium with L-myc RFLP (27). Although we were not able to investigate a linkage disequilibrium pattern between L-myc- Eco R1 and its other sites, it would be valuable to find out the linkage disequilibrium patterns of the L-myc gene.

We also suggest that L-myc RFLP analysis might therefore be an important factor predicting a higher risk for breast cancer, at least in the Turkish population. However, further studies of well characterised large patient groups of defined ethnicity are needed to ascertain the role of S allele in breast cancer.

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