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Investigation of Microsatellite Instability in Turkish Breast Cancer Patients

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Multiple somatic and inherited genetic changes that lead to loss of growth control may contribute to the development of breast cancer. Microsatellites are tandem repeats of simple sequences that occur abundantly and at random throughout most eucaryotic genomes. Microsatellite instability (MI), characterized by the presence of random contractions or expansions in the length of simple sequence repeats or microsatellites, is observed in a variety of tumors. The aim of this study was to compare tumor DNA fingerprints with constitutional DNA fingerprints to investigate changes specific to

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breast cancer and evaluate its correlation with clinical characteristics. Tumor and normal tissue samples of 38 patients with breast cancer were investigated by comparing PCR-amplified microsatellite sequences D2S443 and D21S1436. Microsatellite instability at D21S1436 and D2S443 was found in 5 (13%) and 7 (18%) patients, respectively. Two patients displayed instability at both marker loci. No association was found between MI and age, family history, lymph node involvement and other clinical parameters. (Pathology Oncology Research Vol 8, No 2, 138–141, 2002)

Introduction

Breast cancer is the most prevalent malignancy in women of western industrialized nations.¹ Multiple somatic and inherited genetic changes that lead to loss of growth control may contribute to the development of breast cancer.

Microsatellites are tandem repeats of simple sequences that occur abundantly and at random throughout most eucaryotic genomes.^{2,3} Most of the microsatellites are highly polymorphic due to allelic variation in the repeat copy number in the minisatellites.^{3,5} Different microsatellites have been used to screen a large number of chromosomal loci in the human genome in order to detect somatic changes in various disorders.^{6,7} Chromosomal and genetic instability is a common characteristic associated with the neoplastic phenotype and may lead to tumorigenesis by influencing tumor initiation and progression.⁸ Microsatellite instability (MI) is an early event in tumorigenesis and is characterized by the presence of random

contractions or expansions in the length of simple sequence repeats.⁶ Microsatellite alterations generated during tumor progression have been used as clonal markers of neoplasia. Analysis of the microsatellite instability makes it possible to detect genetic alterations, that may not be detected by the cytogenetics.^{2,9} Presence of microsatellite instability could have clinical implications since *in vitro* studies have revealed that cells with defects in mismatch repair are more resistant to alkylating agents.⁷

Microsatellite instability has been investigated in various types of tumors including colon,¹⁰ bladder,¹¹ gastric¹² and lung¹³ cancer. However, the relevance of microsatellite instability in breast cancer is still under debate.^{14,15} The aim of this study was to investigate the frequency and presence of microsatellite instability in breast tumors and its correlation with clinical and pathological parameters.

Materials and Methods

Fresh tumor and normal tissue samples and peripheral venous blood of 38 patients (mean age: 50.3 ± 14) with breast cancer were investigated. Most patients (n=33) had invasive ductal carcinoma. The normal and tumor tissue

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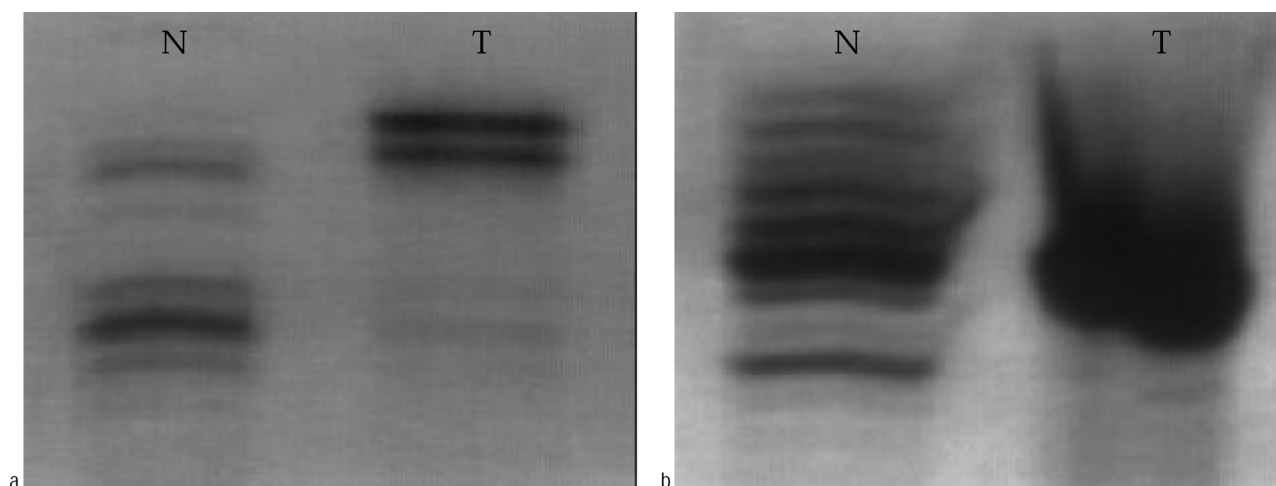


Figure 1. Examples of alterations of microsatellite sequences at (a) D21S1436 and b) D2S443 in breast cancer. T: tumor DNA sample, N: normal DNA sample.

samples were collected during surgery, transferred to the laboratory and were homogenized immediately. DNA was isolated by phenol/chloroform extraction after overnight incubation with proteinase K at 37°C. Tumor and normal DNA were amplified using two different primer pairs specific for known human microsatellites D2s443 and D21s1436. These microsatellite markers on two different chromosomes were selected based on previous studies on breast tumors demonstrating microsatellite instability. Primer sequences (Integrated DNA Technologies, Iowa, USA) were:

GGAA4D07 (5'-GAGAGGGCAAGACTTGAAG-3' and 5'-ATGGAAGAGCGTTCTAAAACA-3'),
GGAA2E02 (5'-AGGAAAGAGAAAGAAAGGAAGG-3' and 5'-TATATGATGAAAGTATATTGGGGG-3')

PCR reactions were performed in 50 µl 1xPCR buffer (Promega) containing 2 mM MgCl₂, 30 µM of each primer, 1-4 µl DNA and 1 U of Taq polymerase (Promega). Amplification was performed using the following conditions: Initial denaturation at 94°C for 4 min., followed by 35 cycles of denaturation at 94°C for 20 seconds, annealing at 55°C for 20 seconds, extension at 72°C for 40

seconds. The reaction was terminated by extension at 72°C for 10 min. PCR products were run on 8% denaturing polyacrylamide gels (8.3 M Urea, 0.5 X TEB Buffer, 4% Glycerol, 1:15 TEMED/10% ammonium-persulphate) and visualized by silver staining.

Statistical significances were calculated by using Fisher's exact test.

Results

The presence of microsatellite instability (MI) at the microsatellite marker loci, D2S443 and D21S1436 was investigated in 38 patients with breast cancer by comparing banding patterns from the normal and tumor samples by PCR. In 10 (26.3%) of 38 patients with breast cancer changes due to the loss and gain of bands or intensity shifts in the banding patterns were observed. In 5 patients (13%) instability at microsatellite D21S1436 was found and in 7 patients (18%) it was observed at microsatellite D2S443 (*Figure 1*). 2 patients displayed instability at both marker loci (*Table 1*). None of the changes in the band pattern were similar among patients with MI.

Table 1. Patients (n=10) who demonstrate instability at microsatellite marker loci D21S1436 and D2S443

TUMORS		B9	B15	B21	B22	B28	B29	B34	B36	B37	B38
MICROSATELLITE MARKER LOCI	D2S443			MI(+)	MI(+)	MI(+)		MI(+)	MI(+)	MI(+)	MI(+)
	D21S1436	MI(+)	MI(+)			MI(+)	MI(+)				MI(+)

No statistically significant association was found between the presence of microsatellite instability and age, lymph node involvement, stage of disease, tumor size, estrogen receptor status, metastasis, family history, histological grade or nuclear grade.

Discussion

Analysis of microsatellite alterations is a new and promising technique to investigate somatic and genetic changes that occur during tumor progression. Using this technique, somatic changes have been reported in different types of human tumors including ovarian, bladder and cervix carcinoma and gastrointestinal system tumors.¹⁶⁻²⁰ In some cancer types correlation between microsatellite instability and stage or prognosis has also been reported.²¹⁻²³ The mechanism how microsatellite instability might induce breast carcinogenesis is still unclear. It is likely that expansion or contraction of microsatellites may affect gene expression and transcriptional regulation leading to breast carcinogenesis.^{23,24} Many repeat sequences have the potential to demonstrate instability.²⁴ Thus an unequivocal indicator which demonstrates instability regions has not been described yet. There are several studies in the literature investigating MI in breast carcinoma at different loci by using different microsatellite markers.^{4,22,25} However, all studies comprise low numbers of patients and the frequency of alterations are quite low for most satellites.^{15,21,24} Thus, using a large number of microsatellites does not necessarily contribute to the diagnostic accuracy. The aim of this study was to compare tumor DNA fingerprints with constitutional DNA and to investigate changes specific to breast cancer. The microsatellite markers used in this study were selected on basis of previous data on breast tumors demonstrating the highest frequencies of microsatellite instability. Instability was observed in 18% of the patients at D2S443 and in 13% of the patients at D21S1436. In two patients instability was present at both of marker loci. These frequencies are lower than a previous report.⁴ However, the frequency of MI in breast cancer and other tumors using the same or different markers is usually much lower in most studies.^{15,22,24,26} Furthermore, in a recent report comprising patients with colon, breast, lung and gynecological cancers no MI has been observed in breast cancer patients using one of the same markers as employed in our study.²⁷ Although a correlation between MI and lymph node involvement, tumor size and stage has been suggested⁴ we did not observe any association between the clinical parameters and presence of MI. Our results are more in line with studies reporting lack of association between microsatellite instability and age,^{4,14,22,24} stage,²⁴ receptor status,^{4,22,24} grade^{14,22} or lymph node involvement.^{22,24} The discrepancy is probably due to the low or even very small^{15,26} numbers of patients enrolled in

the studies. Analysis of a larger cohort of patients and studies to define the optimal panel of microsatellites may resolve this issue.

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