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Investigation of Microsatellite Instability in Turkish Breast Cancer Patients

Semra DEMOKAN,[1] Mahmut MUSLUMANOGLU,[2] YAZİCİ H,[1] Abdullah IGCİ,[2] Nejat DALAY[1]

[1]Oncology Institute, Department of Basic Oncology, [2]Istanbul Faculty of Medicine, Department of Surgery, 34390 Çapa, Istanbul University, Istanbul, Turkey

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 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)

Keywords: microsatellite instability, breast cancer

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 alkalyting agents.[7]

Microsatellite instability has been investigated in various types of tumors including colon,[10] bladder,[11] gastric[12] and lung[13] 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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Correspondence: Prof. Dr. Nejat DALAY, I.U. Oncology Institute, 34390 Capa, Istanbul, Turkey; Fax: 90 212 534 8078; e-mail: ndalay@yahoo.com

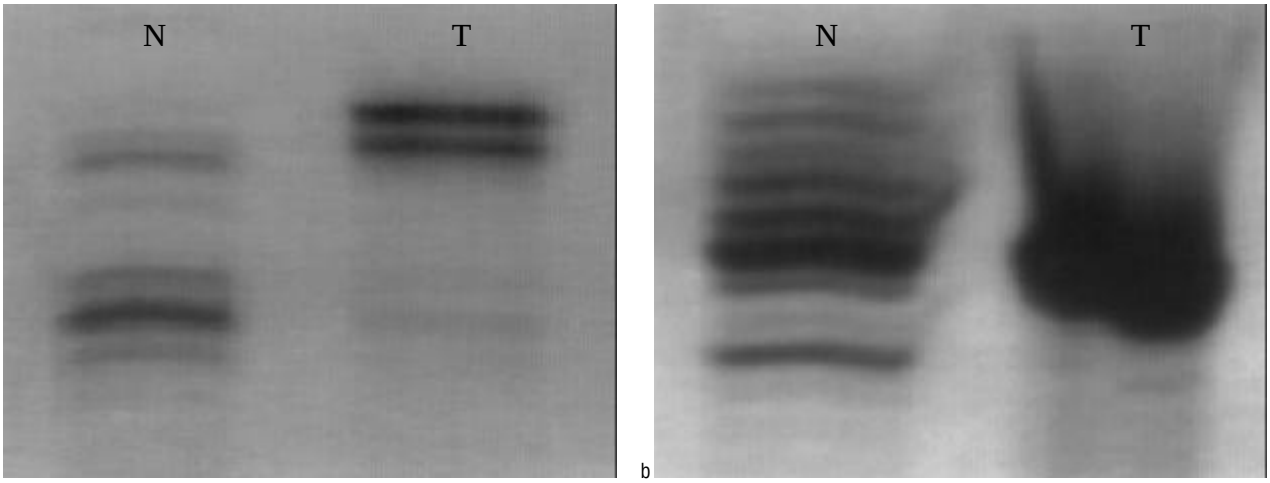

Figure 1. Examples of alterations of microsatellite sequences at (a) D21S1436 and b) D2S443 in breast cancer. T: tumor DNA sample, N: normal DNA sample.

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 37C. 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'-GAGAGGGCAAGACTTGGAAG-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 $MgCl_2$, 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% ammonnium-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 suggested[4] 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[25,26] 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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References

1. *Dickson RB, Lippman ME.* Molecular basis of breast cancer. In: The Molecular Basis of Cancer, WB Saunders, 358-384, 1995.
2. *Hovig E, Smith-Sorensen B, Uitterlinden AG, et al*: Detection of DNA variation in cancer. Pharmacogenetics 2:317-328, 1992.
3. *Paulson TG, Wright FA, Parker BA, et al*: Microsatellite instability correlates with reduced survival and poor disease prognosis in breast cancer. Cancer Res 56:4021-4026, 1996.
4. *Ali S, Müller CR, Epplen JT*. DNA fingerprinting by oligonucleotide probes specific for simple repeats. Hum Genet 74:239-243, 1986.
5. *Decker RA, Moore J, Ponder B, et al*: Linkage mapping of human chromosome 10 microsatellite polymorphisms. Genomics 12:604-606, 1992.
6. *Weber JL, Wong C.* Mutation of human short tandem repeats. Hum Mol Genet 2:1123-1128, 1993.
7. *Branch P, Aquilina G, Bignami M, et al*: Defective mismatch binding and a mutator phenotype in cells tolerant to DNA damage. Nature 362:652-654, 1993.
8. *Fey MF, Wells RA, Wainscoat JS, et al*: Assessment of clonality in gastrointestinal cancer by DNA fingerprinting. J Clin Invest 82:1532-1537, 1988.
9. *Jeffreys AJ, Neumann R, Wilson V.* Repeat unit sequence variation in minisatellites: A novel source of DNA polymorphism for studying variation and mutation by single molecule analysis. Cell 60:473-485, 1990.
10. *Samowitz WS, Curtin K, Ma KN, et al*: Microsatellite instability in sporadic colon cancre is associated with an improved prognosis at the population level. Cancer Epidem Biomarkers Prev 10:917-923, 2001.
11. *van Rhijn BWG, Lurkin I, Kirkels WJ, et al*: Microsatellite analysis – DNA test in urine competes with cystoscopy in follow-up of superficial bladder carcinoma. Cancer 92:768-775, 2001.
12. *Han HJ, Yanagisawa A, Kato Y, et al*: Genetic instability in pancreatic cancer and poorly differentiated type of gastric cancer. Cancer Res 53:5087-5089, 1993.
13. *Gonzalez R, Silva JM, Sanchez A, et al*: Microsatellite alterations and TP53 mutations in plasma DNA of small cell lung cancer patients: Follow-up study and prognostic significance. Annals Oncol 11:1097-1104, 2000.
14. *Contegiacomo A, Palmirotta R, De Marchis L, et al*: Microsatellite instability and pathological aspects of breast cancer. Int J Cancer 64:264-268, 1995.
15. *Vaurs-Barriere C, Penault-Llorca F, Laplace-Marieze V, et al*: Low frequency of microsatellite instability in BRCA1 mutated breast tumors. J Med Genet 37:1-3, 2000.
16. *Chen P, Hurst T, Khoo SK.* Detection of somatic DNA alterations in ovarian cancer by DNA fingerprint analysis. Cancer 67:1551-1555, 1991.

17. *Fearon ER, Vogelstein B*. Tumor suppressor and DNA repair gene defects in human cancer. In : Holland JF, Bast RC, Morton DL, Frei III E, Kufe DW, Weichselbaum RR (eds). Cancer Medicine. 4. ed. Williams and Wilkins, 97-119, 1997.
18. *Alexander J, Watanabe T, Wu TT, et al*: Histopathological identification of colon cancer with microsatellite instability. Am J Pathol 158:527-535, 2001.
19. *Seripa D, Parrella P, Gallucci M, et al*: Sensitive detection of transitional cell carcinoma of the bladder by microsatellite analysis of cells exfoliated in urine. Int J Cancer 95:364-369, 2001.
20. *Rha SH, Dong SM, Jen J, et al*: Molecular detection of cervical intraepithelial neoplasia and cervical carcinoma by microsatellite analysis of Papanicolaou smears. Int J Cancer 93:424-429, 2001.
21. *Gonzalez-Zulueta M, Ruppert JM, et al*: Microsatellite instability in bladder cancer. Cancer Res 53:5620-5623, 1993.
22. *Sourvinos G, Kiaris H, Tsikkinis A, et al*: Microsatellite instability and loss of heterozygosity in primary breast tumors. Tumor Biol 18:157-166, 1997.
23. *Thibodeau SN, Bren G, Schaid D*. Microsatellite instability in cancer of the proximal colon. Science 260:816-819, 1993.
24. *Rush EB, Calvano JE, Van Zee KJ, et al*: Microsatellite instability in breast cancer. Ann Surg Oncol 4:310-315, 1997.
25. *Shaw JA, Walsh T, Chappell SA, et al*: Microsatellite instability in early sporadic breast cancer. Br J Cancer 73:1393-1397, 1996.
26. *Kasami M, Vnencak-Jones CL, Manning S, et al*: Loss of heterozygosity and microsatellite instability in breast hyperplasia. Am J Pathol 150:1925-1931, 1997.
27. *Krajinovic M, Richer C, Gorska-Flipot I, et al*: Genomic loci susceptible to replication errors in cancer cells. Br J Cancer 78:981-985, 1998.