

## **The Role of Allelic Imbalance at Chromosome 2p16 in Breast Cancer Progression**

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### **ABSTRACT**

The role of the mismatch repair gene (MSH2) in breast cancer development was investigated using 9 microsatellite polymorphic markers located at the area of the MSH2 gene on chromosome 2p16. The study included 33 samples of invasive breast carcinoma (IBC), 15 samples of carcinoma in situ (CIS) and 19 samples of epithelial hyperplasia (EH) obtained from 56 patients. There were 11 cases in which more than one pathologic type was obtained from the same patient. Loss of heterozygosity (LOH) was detected in 44.6% of the cases. The highest frequency of LOH was reported in IBC (74.4%) compared to 16.3% in CIS and 9.3% in EH. Microsatellite instability (MSI) was reported in 62.5% of the studied patients, 20.45% in EH, 28.8% in CIS and 51.5% in IBC. There was a highly statistically significant difference in the frequency of MSI and LOH between the three studied groups ( $p = 0.001$ ). Our allelotyping analysis of the 11 cases in which there were more than one lesion type in the same patient revealed that, in 7 cases, aberrations were almost similar in different pathologic lesions obtained from the same patient. In 2 of them MSI in certain markers changed into LOH with disease progression providing evidence for the role of genetic aberrations of MSH2 in the transition of hyperplastic mammary epithelium into neoplastic one. In conclusion, the present study represents the first report that demonstrates the implication of MSH2 gene in the development of sporadic breast carcinoma and its probable precursors. We assume that defects involving the MSH2 gene could promote breast cancer progression through well-defined stages of EH and CIS.

**Key Words:** *Breast carcinoma and precursors - MSH2 - Allelic imbalance.*

### **INTRODUCTION**

Several broad classes of premalignant breast lesions have been defined on the basis of cytological, histological and epidemiological data. These lesions include proliferative breast dis-

ease without atypia and atypical hyperplasia, which are associated with 1.6-4.4 fold-increased risk of subsequent breast cancer development [20]. Carcinoma in situ (CIS) constitutes another group of lesions that confers 8-10 fold increased cancer risk [12]. One model for breast carcinogenesis suggests that the neoplasm develops through the progression of epithelial hyperplasia (EH) to atypical hyperplasia (AH) then in situ and invasive carcinoma [5]. The proofs of this hypothesis came from the observation that 1- in situ carcinomas are frequently present in association with a spectrum of EH at the periphery of large invasive tumors, 2- the greatly increased risk of subsequent development of invasive carcinomas in biopsy-proven CIS and 3- the observation that, when local recurrence occurs after conservative therapy of CIS, there is a 50% chance that the recurrence will be of the invasive variety [17]. However, the events that mediate the transition of normal breast epithelium to premalignant and early tumorigenic states have not been completely determined yet. Numerous studies have documented differences in copy number, sequence or expression levels of certain genes in invasive breast cancer (IBC). An analogous investigation of early breast lesions is still in its infancy, which could be attributed to the small sample size, varying classification systems or the different markers used with different criteria of positivity [20].

Recent studies have focused on the impact of defective mismatch repair (MMR) genes on the pathogenesis of malignancy and genes en-

coding components of the MMR system have been mentioned in relation to several human solid tumors [5,8,18]. Direct evidence for the association of genetic instability and mutated mismatch repair genes is derived from the biochemical studies in vitro in which nuclear extracts from human tumor cell lines with mutated MMR genes are unable to efficiently repair heteroduplex DNA fragments [23]. Hence, it was mentioned that cells with defective MMR mechanisms have a reduction in the fidelity of DNA and cannot correct genetic errors that occur during cellular replication [14]. It has been shown that defects in mismatch repair genes lead to a genome-wide instability of microsatellites which are sequences of the DNA comprising multiple copies of repeat units 1-6 base pairs [3]. When this occurs in protooncogenes or tumor suppressor genes, loss of control over cell growth and proliferation may develop [7].

Microsatellite instability (MSI) was reported in sporadic breast cancer cases suggesting a role for mismatch repair gene defects in breast carcinogenesis [26]. However, the frequency of MSI and loss of heterozygosity (LOH) as a consequence of MMR gene defects in mammary carcinoma varies widely in different studies ranging from 4% to 31% and, consequently, the question whether some breast neoplasms have mismatch repair errors is unresolved yet [7]. In addition, it is still unclear at what stage exactly of breast cancer development the defects in the MMR gene become manifest. The exact timing of occurrence of MMR gene defects may pinpoint their role either in the initiation of tumors or in events that determine subsequent progression assuming that proliferative lesions represent stages in the evolution of breast carcinoma [2]. Moreover, determining the role of MMR gene defects in the pathogenesis of breast cancer and its exact time of occurrence may have significant biological and clinical impact as it may help in understanding the pathogenesis and planning preventive strategies for IBC. The ability to detect alterations in microsatellite loci in clinical samples may be also used as a diagnostic tool for recognizing scanty cancer cells in tumor samples [10] and finally, MSI may prove to be of prognostic significance in breast cancer as in colon cancer and it may also be predictive of tumor's response to certain chemotherapeutic agents [4,9].

Based on these facts, we investigated the involvement of MSH2 gene in sporadic in situ and invasive breast carcinomas as well as in cases with predominant EH in an attempt to find out the role of this gene in the development of breast cancer from its probable precursors.

## MATERIAL AND METHODS

*Tumor samples:* Fresh tumor tissues from patients treated with mastectomy or local excision of breast masses were obtained from the surgical pathology department, NCI, Cairo University. Twenty-seven of these tumors were diagnosed as IBC, 4 as CIS and 10 as EH. In addition, 19 formalin-fixed, paraffin-embedded breast tissues were obtained from American females having breast masses and were diagnosed as 6 cases of IBC, 11 CIS and 9 EH. In 11 cases (7 American and 4 Egyptian cases), there were more than one pathologic type in the same patient from which samples were obtained for DNA analysis (Table 1). In each studied sample, a source of normal tissue was included e.g. normal mammary epithelium, skin or a normal lymph node.

*Microdissection:* of selected populations of normal, hyperplastic, in situ and invasive tumor cells from the paraffin-embedded tissues was performed under direct light microscopic visualization as described by Walsht et al. [24]. All samples were carefully chosen so as to be highly cellular and devoid of necrosis, inflammatory cells or reactive stromal cells.

*Nucleic acid preparation:* Genomic DNA was prepared from paraffin blocks and fresh tumor tissues as described by Zhuang et al. [28]. Microscopic examination of H & E-stained sections from the specimens used for DNA analysis was done prior to the extraction to assure that each sample contains > 80% neoplastic cells.

*Detection of LOH and MSI:* Genetic instability of microsatellite repeat markers and LOH were assessed using a panel of 9 markers that were accurately chosen to cover the entire locus of MSH2 gene at chromosome 2p16. Both extragenic and intragenic markers were used: D2S144, D2S131, D2STOP, D2S177, D2S171, D2S119, D2S136, D2S123 and D2S147.

*PCR for LOH and MSI analysis:* PCR reaction was performed in a 10 µl reaction volume

as previously described [27], then 5  $\mu$ l of the product were electrophoresed on 6% polyacrylamide gel and autoradiographed. Autoradiograms were scored visually and the intensities of the alleles in the tested lesions were compared with those in matched normal tissue.

*Interpretation of the results:* MSI was considered to have occurred if either alleles in the tumor sample showed altered electrophoretic mobility or if new alleles were present in the tumor sample in comparison with the normal. Allelic loss was scored by visual inspection of the autoradiographs. It was considered to occur in the tumor specimens if there was a significant alteration in the relative allele intensities of the tumor sample compared with those from the normal one. Cases showing both LOH and MSI were considered as MSI.

*Control experiments:* To evaluate the reproducibility of the results equivalent amounts of DNA were procured from both normal and lesional cells. Hence, the intensities of amplified alleles were similar in each cell type analyzed. In addition, positive cases were repeated 2-3 times using the same DNA preparations. To consider a case positive for LOH, the same allele should be lost in each repeat experiment.

*Statistical analysis:* Statistical program for Social Science (SPSS) package was used for data analysis. Chi square and Fisher exact tests were used to compare proportions.  $p$  value is always two-tailed and considered significant at 0.05 level.

## RESULTS

The role of the mismatch repair gene (MSH2) in breast cancer development was investigated using 9 microsatellite polymorphic markers located at chromosome 2p16. Both extra- and intragenic markers were used to cover the whole area of the MSH2 gene. The study included 33 samples of IBC, 15 samples of CIS and 19 samples of EH obtained from 56 patients having breast lesions. Out of the 76 samples analyzed, 26 were obtained from American females.

A total of 603 reactions were performed and 532 of them were considered informative. Both LOH and MSI were scored, however cases showing both aberrations were considered non-

informative for LOH. Tables (2-5) show the results of testing at all loci.

The frequency of allelic imbalance (AI) as a whole (MSI & LOH) was 17.7% (31/175) in EH, 25.7% (45/175) in CIS and 56.6% (99/175) in IBC. The highest frequency of AI was detected at D2S171 in all studied groups constituting 14.4% (19/132) of MSI and 27.9% (12/43) of LOH.

Forty-three instances of LOH were detected out of the 175 (24.6%) informative instances that revealed AI in any of the studied groups. They were all clustered in 25 out of the 56 patients examined giving a 44.6% overall incidence of LOH in breast cancer and its probable precursors. In 12 cases [11 IBCs and a single case of CIS (CIS13)], LOH was detected in more than one marker.

Only 4 out of the 43 instances of LOH (9.3%) were reported in case of EH and all were clustered at D2S171. As for CIS, 7 instances (16.3%) of LOH were reported compared to 32 instances (74.4%) in cases of IBC. The highest frequency of LOH in CIS was reported at D2S171 and D2STPO (2 instances each), whereas in case of IBC the highest frequency was reported at D2S171 (6 instances). Although there was no statistically significant difference between the 9 studied markers regarding the frequency of LOH, the difference in the frequency of LOH between the 3 studied groups was highly statistically significant ( $p = 0.001$ ).

There were 132 instances (75.4%) of MSI reported in this study. Out of the 56 patients examined, 35 (62.5%) revealed MSI in at least one of the studied markers (Fig. 1). However, the majority of cases (21/35) showed MSI in more than one marker. Twenty seven out of 132 instances of MSI were reported in EH (20.4%) compared to 38 (28.8%) and 67 (50.8%) in CIS and IBC, respectively. The highest frequency of MSI reported in this study was at D2STPO and D2S177 in EH (4 instances each), at D2S131, D2S123 and D2S171 in CIS (6 instances each) and at D2S171 in IBC (10 instances). The difference in the frequency of MSI between the three studied groups was statistically significant ( $p = 0.001$ ), being highest in the group of IBC (50.8%) versus 28.8% and 20.5% in CIS and EH, respectively.

In the present work, there are 2 cases in which MSI of a certain allele changed into LOH with disease progression. In T10, D2S119 and D2S177 showed MSI in samples obtained from the areas of EH. However, the DNA obtained from areas of IBC in the same patient showed LOH for both markers (Tables 2 & 4). This was also observed in CIS14 in which, LOH at D2STPO was detected in CIS whereas DNA from areas of EH obtained from the same patients showed MSI at the same marker (Tables 2 & 3). On the other hand, in 6 cases in-

cluding the 2 previously mentioned ones (T3 & CIS3, T4 & EH4, T10 & EH10, T31 & CIS12, T33 & CIS13, CIS14 & EH19), almost the same aberrations were detected in lesions obtained from the same patient (Fig. 2).

Cases number T15 and T18 revealed aberrations in all studied markers, whereas no aberration was detected in any of the studied markers in cases number T1, T5, T8, T9, T11, T16, T20, T28, T29, CIS5, CIS6, CIS7, CIS8, CIS9, EH1, EH8, EH9, EH11, EH13, EH17, EH18.

Table (1): Cases showing more than one pathological type in the same patient.

Case No.	D2S144	D2S131	D2STPO	D2S177	D2S171	D2S119	D2S136	D2S123	D2S147
CIS3	◦	◦	◦	◦	◦	NV			◦
T3	◦	◦	◦		◦	◦		◦	◦
T4			NV		NV	◦			
EH4						◦			
CIS5									NV
EH12					◦				
CIS6		NV		NV					NV
EH13		NV		NV					NV
CIS7					NV				NV
EH17					NV				NV
CIS11		◦		NV	◦	◦	◦	◦	NV
T30		NV		NV	◦				NV
CIS12		NV		NV	•	NV	NV	◦	NV
T31		NV			NV	NV	NV	◦	NV
CIS13		NV	•			•	•		NV
T33	◦	NV	•		•		•	◦	NV
CIS14	NV	NV	•			NV		◦	NV
EH19			◦			NV		◦	NV
EH8									
T8									
EH10			◦	◦	•	◦	◦		◦
T10	◦		◦	•	•	•			◦

• LOH    ◦ MSI    NV: Not valid    T: Tumor    CIS: Carcinoma in situ    EH: Epithelial hyperplasia.

Table (2): Allelic imbalance at different markers in each pathological type.

Marker	EH			CIS			IBC		
	INF	LOH	MSI	INF	LOH	MSI	INF	LOH	MSI
D2S144	19	0	3 (15.8%)	14	1 (7.1%)	4 (28.6%)	33	3 (9.1%)	10 (30.3%)
D2S131	17	0	3 (17.6%)	10	0	6 (60%)	29	2 (6.9%)	7 (24.1%)
D2STPO	19	0	4 (21.1%)	15	2 (13.3%)	4 (26.7%)	32	3 (9.4%)	8 (25%)
D2S177	15	0	4 (26.7%)	10	0	4 (40%)	31	4 (12.9%)	7 (22.6%)
D2S171	18	4 (22.2%)	3 (16.7%)	14	2 (14.3%)	6 (42.85%)	30	6 (20%)	10 (33.3%)
D2S119	17	0	3 (17.6%)	11	1 (9.1%)	3 (27.3%)	28	5 (17.85%)	6 (21.4%)
D2S136	19	0	1 (5.3%)	13	1 (7.7%)	2 (15.4%)	32	4 (12.5%)	5 (15.6%)
D2S123	19	0	3 (15.8%)	15	0	6 (40%)	32	3 (9.4%)	7 (21.9%)
D2S147	10	0	3 (30%)	4	0	3 (75%)	26	2 (7.7%)	7 (26.9%)

T: Tumor      CIS: Carcinoma in situ      EH: Epithelial hyperplasia.

Table (3): Allelic imbalance at the MSH2 gene in cases of epithelial hyperplasia.

	D2S144	D2S131	D2STPO	D2S177	D2S171	D2S119	D2S136	D2S123	D2S147
EH1	◦	◦	◦		◦			◦	◦
EH2	◦	◦	◦		◦	◦		◦	◦
EH3						◦			◦
EH4						◦			
EH5				◦					
EH6				◦					
EH7	◦	◦		◦	•	◦			
EH8									
EH9									
EH10			◦	◦	•				◦
EH11									NV
EH12					◦				NV
EH13		NV		NV					NV
EH14				NV					NV
EH15				NV	•		◦		NV
EH16		NV		NV	•	NV			NV
EH17					NV				NV
EH18									NV
EH19			◦			NV		◦	NV

Cases number 11-19 were obtained from American patients.

• LOH      ◦ MSI      NV: Not valid      INF: Informative.

Table (4): Allelic imbalance at the MSH2 gene in cases of carcinoma in situ.

	D2S144	D2S131	D2STPO	D2S177	D2S171	D2S119	D2S136	D2S123	D2S147
CIS1	◦	◦	◦	◦	◦	◦	◦	◦	◦
CIS2	◦	◦	◦	◦	•	◦		◦	◦
CIS3	◦	◦	◦	◦	◦	NV			◦
CIS4	◦	◦	◦	◦	◦				◦
CIS5									NV
CIS6		NV		NV					NV
CIS7					NV				NV
CIS8									NV
CIS9									NV
CIS10		◦		NV		◦			NV
CIS11		◦		NV	◦	◦	◦	◦	NV
CIS12		NV		NV	•	NV	NV	◦	NV
CIS13		NV	•			•	•		NV
CIS14	NV	NV	•		◦	NV		◦	NV
CIS15	•	NV		NV	◦	NV	NV	◦	NV

Cases number 5-15 were obtained from American patients.

• LOH      ◦ MSI      NV: Not valid

Table (5): Allelic imbalance at the MSH2 gene in cases of invasive breast cancer.

	D2S144	D2S131	D2STPO	D2S177	D2S171	D2S119	D2S136	D2S123	D2S147
T1									
T2				•					
T3	°	°	°		°	°		°	°
T4			NV		NV	°			
T5									
T6				°					
T7	°	°	°	•	°	°	°		•
T8									
T9									
T10	°		°	•	°	•	°		°
T11	°	°	•		•	°	°	°	°
T12	°								
T13	°					•			
T14								°	
T15	°	°	°	°	°	•	°	°	°
T16									
T17		°		°		•			
T18	°	°	°	°	•	°	•	•	°
T19		°	°			•		•	•
T20									
T21	•				°	°	•		
T22	•		°	°	°		°		NV
T23			°	°	•				°
T24	•	•			°				°
T25	°	•	•	°	°			°	
T26					•	NV			
T27					°	NV	•	•	NV
T28						NV			
T29		NV		NV	NV				NV
T30		NV		NV	°				NV
T31		NV		NV		NV	NV	°	NV
T32	°			•	•	NV		NV	NV
T33	°	NV	•		•		•	°	NV

Cases number 28-33 were obtained from American patients.

• LOH      ° MSI      NV: Not valid

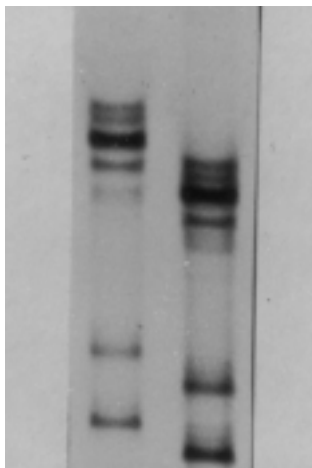


Fig. (1): An example of microsatellite instability at D2S144 in a case of pure carcinoma in situ (CIS 4). Lane 1 represents normal and lane 2 represents tumor (CIS) DNA.

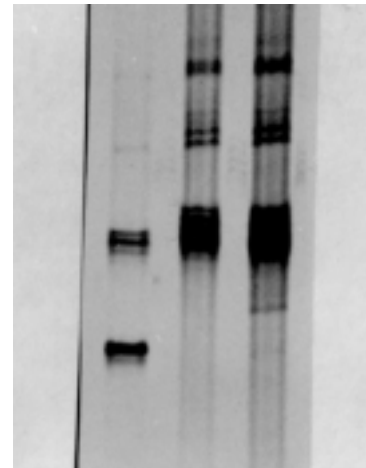


Fig. (2): A case of carcinoma in situ (CIS 12) and invasive breast carcinoma (T31) obtained from the same patient. Both lesions showed microsatellite instability at D2S123. Lane 1 represents normal, lane 2 represents CIS 12 and lane 3 represents IBC-T31 DNA.

## DISCUSSION

A proposal that deficient DNA repair is a predisposing factor in breast cancer was prompted by reports of MSI in sporadic breast tumors [13,15]. However, many of the previous reports of AI in breast cancer are inconsistent with one another. Several laboratories have reported AI in early stage breast cancer [19,22]; others reported AI principally in advanced cases [1,15], whereas some investigators failed to detect any AI in any of their studied cases [12]. It was shown in a previous study on Egyptian breast cancer patients that AI at the area of MSH2 gene is a common feature in IBC, being detected in 22 out of 29 studied cases of IBC cases [27]. Therefore, in the present study, the work was extended to include cases of CIS and EH in an attempt to investigate the role of MSH2 gene defects in sporadic breast cancer and to determine at what stage exactly do they become manifest.

The present study provides evidence that MSI at the area of MSH2 gene is an important feature of breast carcinoma and its probable precursors, being detected in 62.5% of the tested cases. This disagrees with the assumption provided by Dillon [7] who mentioned that MSI is not a significant feature of breast cancer or any of its precursors and consequently, it is not an important cause or effect of the processes leading to the development of these pathological states. The frequency of MSI in the 3 studied groups was 62.5%, 20.4% in EH; 28.8% in CIS and 50.8% in IBC, which is comparable to Dillon et al. [7] in the three studied groups. They reported 33%, 32% and 65% in EH, CIS and IBC, respectively. The similarity in the results between both studies in spite of the difference in the markers used indicates that several genes and chromosomal loci should be involved in the progression of breast carcinoma through the stages of hyperplasia and CIS, [16].

On the contrary, our results are inconsistent with those of Wooster [25], Dietmaier [6] and Benachenhou [3] who failed to find any MSI at the area of MSH2 gene. However, the latter were able to detect LOH in 5% of their 22 cases only. These conflicting data could be attributed to 1- the larger number of analyzed samples in our study and the larger number of markers used, 2- the difference in the location of the studied markers, 3- the inclusion of CIS and EH

cases, 4- sample stratification, or it may require another explanation.

Anbazzhagan [2] also failed to find any MSI in any of their 267 sporadic breast cancer cases using a panel of 104 markers including D2S123 and BAT26 at 2p16-21.

They explained their negative results by the possibility that defects in MMR genes studied could have occurred in some breast cancer cells without subsequent clonal expansion and therefore they escaped detection. Consequently, they assumed that MSI is not significant to the pathogenesis of IBC. We disagree on this assumption as failure to detect genetically aberrant cells could be attributed to the low sensitivity of their technique due to improper PCR conditions rather than to the absence of clonal expansion because when they repeated 8 of their 10 positive cases, no MSI was detected in the repeat experiments in any of the cases.

The data provided in this study indicate that, MSI develops at an early stage in the genetic cascade of breast cancer. It was first observed at low level (19.7%) in cases of EH indicating that a proportion of the hyperplastic cells started to accumulate the genetic alterations. The rate was similarly low in CIS (28.8%) but it increased sharply to 51.5% in invasive tumors. However, the difference between the three studied groups was not as high as it is in case of LOH. Therefore, it could be assumed that MSI is a common genetic event that starts to be manifest at an early stage of breast carcinogenesis.

Our results regarding MSI in the group of CIS patients are consistent with that of Walsh [24] who found MSI at multiple chromosomal loci in 22% of their cases. Using immunohistochemical techniques, inactivating mutations of the MSH2 gene were detected as decreased expression of the protein product or as expression of a truncated protein not detected by the antibodies used.

We were able in this study to show that, LOH at the area of MSH2 gene occurs concurrently with the development of invasive carcinoma indicating that LOH is a late event in breast carcinogenesis which is usually associated with the acquisition of the invasive phenotype. Previous reports demonstrate a frequency of 19-60% at different chromosomal loci [6,21]. In the present study, the overall incidence of

LOH in the 3 studied groups was 24.6% which is comparable to that reported by Dillon [7] who reported a frequency of 24-38% at several chromosomal loci. Although the markers used in the two studies were completely different except for a single marker (D2STPO), the similarity in the results could give an overview for the incidence of LOH in sporadic breast cancer and its probable precursors. The present results are also consistent with that of Zekri [27] and Radford [11] who were able to detect LOH at MSH2 in 26% and 30% of their breast cancer patients, respectively.

Our results regarding LOH in EH and CIS are consistent with those of Dillon [7] who reported LOH in 8% and 11.9% of the cases, respectively. However, our results of LOH in CIS patients are much lower than that of Aldaz [1], who found LOH in 74% of 23 CIS cases using a panel of 20 markers. This discrepancy could be attributed partially to the difference in the location of the markers used and partially to the large number of markers they had used in their study which enabled them to detect more aberrations. They are also inconsistent with those of Benachenhou [3] who had shown in their study that, LOH is not a common feature of IBC being detected in 5% only of 22 studied cases of sporadic breast cancer. The higher frequency reported in our study could be attributed to the strict criteria of sample selection. In the present study, all samples were accurately chosen so that, each sample contained more than 80% neoplastic cells. This was assured by using the microdissection technique to get the areas of highest cellularity or by examination of H & E-stained sections of analyzed specimen prior to DNA extraction. This step was useful in minimizing the possible masking of allelic losses that could result from contaminating genetic material of normal cells. In addition, the polymorphic markers used by Benachenhou [3] are different and fewer than those used in the present study with a possible loss of some aberrations that may be present outside the area covered by their markers.

The observation that D2S171 was the only marker showing LOH in the group of EH could provide an evidence that this marker represents the first locus of the MSH2 gene to be affected in the process of breast carcinogenesis. This indicates that it is a possible candidate that could be used for the prediction, early detection and

follow up of a group of patients with proliferative breast disease who are prone to develop invasive breast carcinoma. In addition, D2S171 revealed the highest frequency of aberrations in CIS and IBC. This clearly demonstrates the importance of this locus at least in breast carcinogenesis. In this context, Dillon [7] mentioned that, in particular tumor types, certain microsatellite loci are inherently more likely to show instability than others even though the repeat length is the same (tumor-specific instability). This tumor-specific instability could also explain the discrepancy in results between different research groups. Consequently, Mao [10] described a method for detection of primary bladder cancer using 60 markers against 50 cases of primary bladder cancer. At the end, they were able to select a panel of 10 markers to be used for screening of bladder cancer. Our results demonstrate also that D2S171 is the first marker to show aberrations at the area of MSH2 in the process of breast carcinogenesis.

In conclusion, the present study represents the first report that demonstrates the implication of MSH2 gene in the development of sporadic breast carcinoma and its probable precursors. We were able to show that defects involving the MSH2 gene start to occur at the stage of EH and increase through the stages of in situ and invasive carcinomas. Consequently, we assume that defects in MSH2 gene could promote breast cancer progression through the stages of EH and CIS. Our allelotyping analysis of the 11 cases in which there were more than one lesional type obtained from the same patient provides additional evidence for the proposed model of breast cancer development through well defined stages of EH and CIS. The observation that the genetic aberrations were almost similar in different pathologic lesions obtained from the same patient, and that in certain cases MSI of some markers changed into LOH with disease progression, provides additional evidence for the role of genetic aberrations at the MSH2 gene in the transition of a hyperplastic mammary epithelium into a neoplastic one.

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