

## **Frequent Allelic Losses at the Short Arm of Chromosome 3p in Invasive Ductal Carcinoma of Breast in Egypt**

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### **ABSTRACT**

Breast cancer is one of the most common cancers affecting women in western countries as well as in Egypt. Deletion on the short arm of chromosome 3 (3p) has been detected in breast cancer using cytogenetic and loss of heterozygosity (LOH) studies which suggested the presence of tumor suppressor gene loci on that chromosomal arm. In this report 45 cases of invasive ductal carcinoma of breast were studied for LOH using 10 highly polymorphic microsatellite markers distributed over the short arm of chromosome 3 (3p). LOH was observed in 87% of the cases examined. In this study, three common regions on 3p chromosome showed LOH. The highest LOH was detected at the 3p14-13 region which showed 42.75% of allelic losses. The second region was at 3p14.2-14.1 where 21% of allelic losses were observed. This region contains the recently identified Fragile Histidine Triad (FHIT) tumor suppressor gene. The last region that showed LOH was at 3p21.1-14.3 region which exhibited 21% losses. No significant correlation was observed between LOH and different clinico-pathological parameters. These results suggest that 3 or more tumor suppressor gene regions on the short arm of chromosome 3 including the FHIT gene may play an important role in breast cancer tumorigenesis.

**Key words:** *Allelic loss - Chromosome 3 - Breast cancer*

### **INTRODUCTION**

Breast cancer is one of the most common neoplasms affecting women of western countries. In North America, breast cancer is the most common malignancy among women and accounts for 27% of their cancers. Eighteen per cent of female cancer deaths are due to breast cancer [28]. In Egypt, data reported by Mokhtar [14] indicated that breast cancer ranked as number one (27.3%) among carcinoma of females. In the Nordic countries, Ewertz [6] reported that it is the most common malignant disease among women and the incidence has been increasing steadily for the past 30 years.

Breast cancer is recognized by histological

studies to progress through a series of in situ stages prior to the development of infiltrating and metastatic cancer, which develops and progresses through a sequence of events involving the activation of oncogenes and inactivation or loss of tumor suppressor genes [21]. Loss of heterozygosity (LOH) analysis was used in studies of most types of cancer, and is considered to be the basis of subsequent investigations to identify and clone the genes involved in tumor development. The molecular changes responsible for progression to an advanced stage of the disease are not yet well understood. Some studies on LOH have implied different chromosomal regions that are most frequently involved [1,4,7]. Deletions on the short arm of chromosome 3 (3p) have been detected in many human malignancies including breast cancer [5,13,21]. It is a broad common region, suggesting that at least three tumor suppressor gene loci are suspected to be present on it. One of the tumor suppressor genes identified on chromosome 3p is the VHL (Von Hippel Lindau disease) gene at 3p25 which was found to play a role in the tumorigenesis of renal cell carcinoma and hemangioblastoma [11]. The human FHIT (Fragile Histidine Triad) gene was identified and localized at 3p14.2 and was found to be deleted in different types of cancer including breast cancer [8,9,25].

The present study was aimed out to search for potential tumor suppressor genes on the short arm of chromosome 3 (3p) in a set of 45 invasive ductal carcinomas of the breast by identifying a common region of deletion using 10 highly polymorphic microsatellite markers and to find whether or not a correlation exists between deletion of 3p with various clinical features including histopathologic grades and lymph node status.

## MATERIAL AND METHODS

### *Patients and samples:*

Tissues from primary breast tumors and their matching normal tissues or blood lymphocytes were obtained from 45 patients that underwent either radical mastectomy or lumpectomy at the National Cancer Institute, Cairo, Egypt. The age of patients ranged from 30 to 75 years with the mean of 47.2 years. Twenty women were under 50 years and 25 were 50 years of age or over. The tumors were histologically classified according to the criteria of the World Health Organization [17] and staged according to the criteria of the American Joint Committee on Cancer [2]. All the pathological data were collected from the department of pathology, NCI, Cairo University. All tumors were classified as invasive ductal carcinomas, 29 (64.4%) were grade 2 and 16 (35.6%) were grade 3. Meanwhile, 38 of the analyzed tumors were positive for lymph node involvement. All patients had received neither chemotherapy nor radiotherapy prior to surgery.

### *Allelic losses Analysis:*

High molecular weight DNA was extracted from tissues and blood lymphocytes by proteinase-K digestion followed by phenol/chloroform extraction and precipitation by ethanol as previously described [20].

Analysis of allelic losses of chromosome 3p was performed on DNA extracted from tumor and matching normal tissues or lymphocytes using 10 microsatellite loci distributed along chromosome 3p. The markers used in this study were dinucleotide CA-repeats. All primers were obtained from Life Technologies (GIBCO-BRL) and the sequences were obtained as described before [16]. Polymerase chain reaction (PCR) was performed in a total volume of 10  $\mu$ l, containing 20 ng of genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5mM MgCl<sub>2</sub>, 200 mM each of four dNTPs, 10 mM of each primer, 1 uCi (-<sup>32</sup>p)dCTP (3000 Ci/mmol, Amersham, Arlington Heights, AL) and 0.25 U of Taq DNA polymerase. The PCR reaction was programmed as follows: initial denaturation, 5 min. at 95°C, amplification, 1 min. at 94°C, annealing 1 min at 55-62°C, extension 1 min at 72°C for 35 cycles. Final elongation for 10 min at 72°C was allowed. PCR products were processed by adding 10  $\mu$ l of loading buffer (containing 95% formamide, 0.05% bromophenol blue, 0.05% xylene cyanol, and 20 mM EDTA). The reaction was denatured at 95°C for 5 min and chilled in ice, 5  $\mu$ l was loaded onto 6% acrylamide containing 7M urea in

IXTBE buffer for 2-3 hours at 60W at room temperature. The gels were dried and exposed to a Kodak X-ray film at -80°C for 1 or 2 days.

The samples were considered informative for each particular chromosomal marker if two alleles could be identified with normal DNA. Allelic loss (LOH) was scored if the autoradiographic signal of one allele was approximately 60% reduced in the tumor DNA compared with the corresponding normal allele. The evaluation of the signal was determined by visual examination by three independent viewers.

### *Statistical methods:*

Statistical analysis for correlation of genetic changes (LOH) with clinicopathological characteristics were performed using Chi-square test. A *p*-value of <0.05 was considered to be statistically significant.

## RESULTS

In order to determine the frequency of allelic loss at the short arm of chromosome 3 (3p) in invasive ductal carcinoma of the breast, 10 highly polymorphic markers distributed along the 3p region were used. The markers used in our study were informative in a mean of 86% of cases with a range of 58% (D3S1067) to 91% (D3S659). The overall LOH was observed in 87% (39 out of 45) of cases examined for at least one or more of the 10 loci used. The majority of tumors with LOH showed reduced intensity of one allele which was interpreted as normal tissue contamination in the tumor. Complete loss of an allele was observed in few tumors.

In the present study, more than one region on chromosome 3p revealed loss of heterozygosity. The most common region that showed the highest percentage was the region between 3p14-13 which has both D3S1210 and D3S659, where 42.75% of allelic losses were detected in this region. Out of the 41 informative tumors, 20 (48.9%) revealed LOH at locus D3S1210 located at p14-13. Fig. (1-A) shows deletion of the lower allele in tumor cases #5 and 8 as compared to normal of the same patient. Locus D3S659, which lies at 3p13, also exhibited high frequency of LOH, where out of 41 informative cases, 15 (36.6%) exhibited LOH. Fig. (1-B) shows deletion of the lower allele in tumor cases #15,21 and 35. The second region that exhibited LOH was located at 3p14.2-14.1, where 21% allelic loss out of the informative tumor

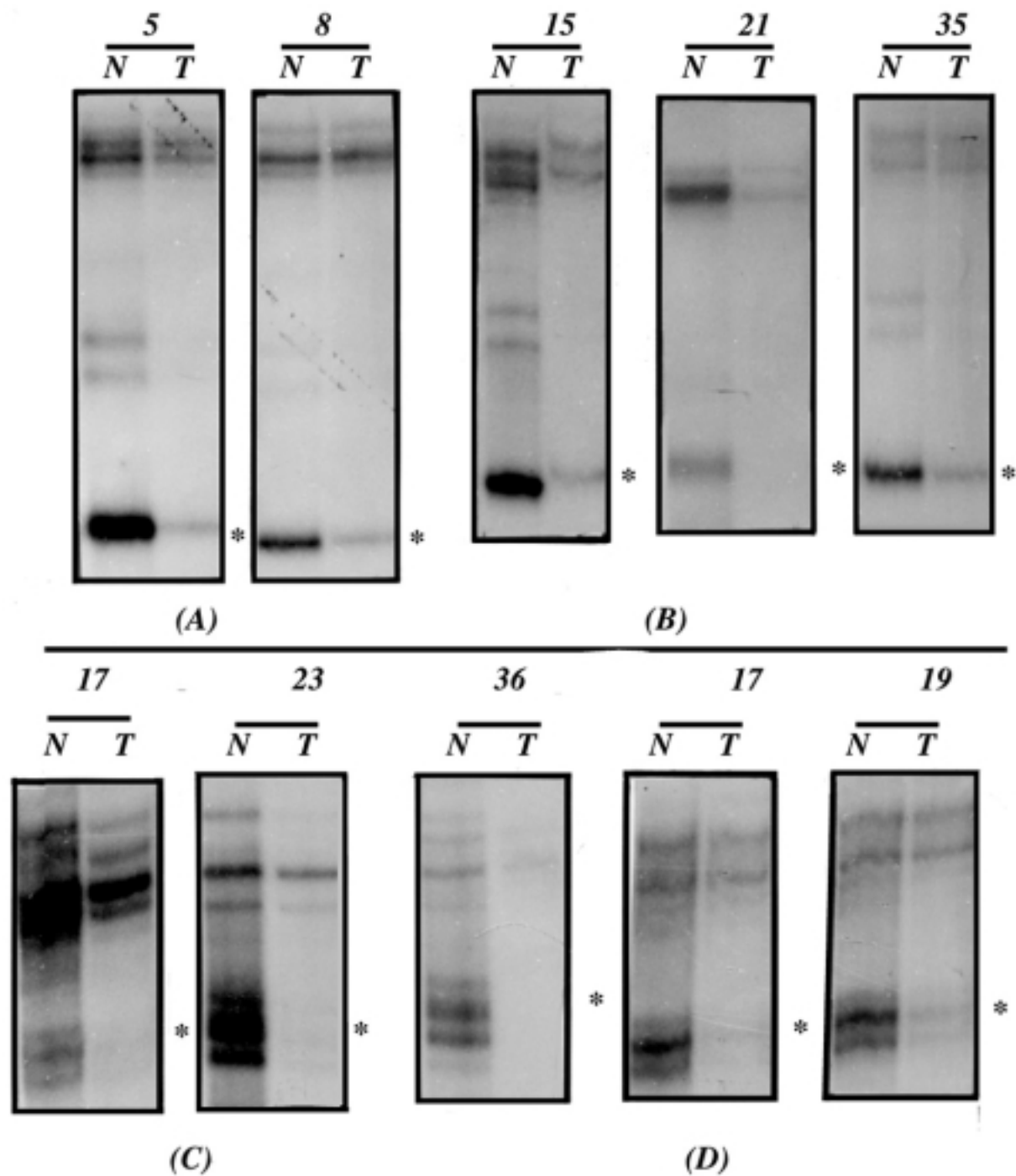


Fig. (1): Loss of heterozygosity (LOH) of chromosome 3p in invasive ductal carcinoma of breast from genomic DNA derived from normal (N) and tumor (T) tissues of the same individuals.

Fig. (1-A) shows LOH for marker D3S1210,

Fig. (1-B) shows LOH for marker D3S659,

Fig. (1-C) shows LOH for marker D3S1285; and

Fig. (1-D) shows LOH for marker D3S1067.

\* indicates the position of allelic loss.

analyzed exhibited LOH using marker D3S1285. Fig. (1-C) denotes deletion of the lower allele in cases #17, 23, and 36 for locus D3S1285. The third region that exhibited LOH in our study was located at p21.1-14.3, where 21% and 23% revealed allelic losses of the informative tumors analyzed in the loci D3S1289 and D3S1067, respectively. In Fig. (1-D), a deletion in the lower allele of the cases #17 and 19 for marker D3S1067 was detected.

D3S1317, D3S1100, not 73, and D3S1235, which are located between the region p26 to 21.1, showed infrequent allelic losses, where 9.7%, 15.4%, 15%, and 12.2% of LOH were observed for the previously stated loci, respectively. Also, locus D3S1251 which lies at 3p12-cent exhibited 17.5% LOH of the informative tumors analyzed (Table 1). In the present study, no whole deletions on chromosome 3p were observed. One tumor only (case #12) revealed LOH at 4 loci, whereas 10 tumors revealed LOH at 2 loci, 7 cases exhibited LOH at one locus only and in 9 cases no LOH could be detected at any of the 10 loci examined. The order of the 10 microsatellite loci analyzed in this study, their location on chromosome 3p, the number of informative cases, and the number of LOH are presented in table (1).

There was no significant correlation between LOH at 3p chromosome with different clinico-pathological parameters including tumor grade. Also, the correlation between 3p LOH with lymph node status showed that 84% of the cases examined that exhibited LOH were positive for lymph node metastasis, whereas 57% of lymph nodes were free of metastasis. However, this correlation is still not statistically significant  $p > 0.05$ ).

Table (1): Summary of allelic loss (LOH) for 10 microsatellite markers in invasive ductal carcinomas of breast.

Microsatellite Markers	Chromosomal region	No. of informative cases (%)	No. of LOH (%)
D3S1317	3p26	41/45(91)	4/41(9.8)
D3S1100	3p24.2-21.3	39/45(87)	6/39(15.4)
not 73	3p23-21.3	40/45(89)	7/40(17.5)
D3S1235	3p21.3-21.1	41/45(91)	5/41(12.2)
D3S1289	3p21.1	38/45(84)	8/38(21.1)
D3S1067	3p21.1-14.3	26/45(58)	6/26(23.1)
D3S1285	3p14.2-14.1	38/45(84)	8/38(21.1)
D3S1210	3p14-13	41/45(91)	20/41(48.8)
D3S659	3p13	41/45(91)	15/41(36.6)
D3S1251	12-cent	40/45(89)	7/40(17.5)

## DISCUSSION

A dramatic increase in the knowledge of the genetic changes occurring in human cancer cells has been known over the past few years. This progress is largely due to the development of techniques such as polymerase chain reaction (PCR). Loss of heterozygosity (LOH) in tumor samples is believed to be a marker for the absence of a functional tumor suppressor gene. Non random chromosome deletions and LOH at specific chromosomal regions were identified in a number of common human cancers including carcinoma of the breast [10]. Molecular and cytogenetic analysis with highly polymorphic microsatellite markers have delineated three distinct regions of common allelic loss at chromosome 3p that have been observed in different types of tumor, including renal cell carcinoma [15], ovarian [5], testicular [12], and breast [19] tumors.

In all regions studied, the region 3p14-13 showed the highest deletion detected in this study where marker D3S1210 exhibited 48.9% LOH and marker D3S659 exhibited 36.6% LOH. These data are consistent with the previous studies that identified a common region of deletion in ovarian and renal cell carcinomas and other malignancies at 3p13-14.3 region [22,29]. Also, a cytogenetic study observed a deletion at 3p13-14 region as a primary change in breast carcinogenesis [19].

The second region that exhibited LOH was the region 3p14.1-14.2, which is detected in this study by marker D3S1285, showed 21% allelic loss. This region contains FRA3B, the most frequently inducible fragile site in the human genome [24] and its biological significance may be due to its potential involvement in several malignancies. Recently, the fragile histidine triad gene (FHIT) has been identified at 3p14 and shown to span a renal carcinoma-associated translocation breakpoint [18]. Abnormalities in FHIT have been identified also in digestive tract cancers [18] and lung cancer [25,27]. Furthermore, homozygous deletion in FRA3B region was observed in some tumor cell lines [26]. A previous study observed LOH at FHIT region in tumors of the breast, which may reflect the importance of FHIT in the development of sporadic breast cancer [3]. The present data are consistent with those results which revealed the involvement of FHIT region in sporadic breast carcinoma.

The third region that exhibited LOH was 3p21 region, 21% and 23% for markers D3S1289 and D3S1067 were observed, re-

spectively. LOH was observed at 3p21 in many common cancer types besides the presence of homozygous deletion in lung and breast cancers [25]. Another study presented 41% of LOH at the 3p21 region in esophageal squamous cell carcinoma [23]. This finding indicates that the 3p21 region is another important region in the development of several common sporadic cancers including breast.

Marker D3S1317, which lies very close to von Hippel-Lincoln (VHL) gene, exhibited only 9.7% allelic loss, which explains that VHL gene is not involved in the development of breast carcinoma. Also, markers D3S1100, not 73, and D3S1235 which lie at 3p24.3 and 3p21.1 showed infrequent percentage of LOH.

No significant correlation was observed in this study between LOH at 3p with any of the clinico-pathological parameters analyzed such as histopathological type and grade. For lymph node metastasis, 84% of the cases denoting LOH were positive for lymph node metastasis and 43% were negative and these changes were still not statistically significant.

In conclusion, we can conclude from the present study that there are 3 or more tumor suppressor genes at 3p14-13, 3p14.2, and 3p21 which may be involved in the development of ductal cell carcinoma of the breast. However, more studies are needed to clarify the exact stage at which they act.

#### Acknowledgment

The author would like to thank Dr. Mohammed Abul Hassan, Assistant Lecturer in cancer biology department, NCI, for his technical assistance; and the department Pathology, NCI, for supplying the materials and clinical data of the cases examined.

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