

## RESEARCH COMMUNICATION

# Comparative Genomic Hybridization (CGH) Analysis of Chromosomal Aberrations in Iranian Patients with Invasive Ductal Carcinoma Breast Cancer

Saeed R Ghaffari<sup>1-4\*</sup>, Tayebeh Sabokbar<sup>1</sup>, Peyman Noshiravan Pour<sup>5</sup>, Jila Dastan<sup>4</sup>, Farhad Mehrkhani<sup>1</sup>, Solmaz Shoraka<sup>1</sup>, Mohammad Ali Mohagheghi<sup>6</sup>, Farrokh Tirgari<sup>5</sup>, Alireza Mosavi-Jarrahi<sup>7</sup>

### Abstract

**Introduction:** Breast cancer is one of the most common cancers in women; however, due to the complexity of chromosomal changes, limited data are available regarding chromosomal constitution. **Materials and Methods:** In this study, Comparative Genomic Hybridization (CGH) was used on 16 Iranian patients diagnosed with invasive ductal breast carcinomas. **Results:** 12 samples had abnormal CGH results (75%), including 21 types of chromosomal imbalance. The most prevalent were chromosomal gain of +1q, +17q, +8q and chromosomal loss of -13q. All three cases with DNA loss at chromosome 13q (-13q) had lymph node metastasis. **Conclusions:** CGH is able to detect chromosomal abnormalities which are difficult to identify by conventional cytogenetic techniques. More studies on a larger sample size may help to confirm or rule out any possible correlation between 13q monosomy and lymph node metastasis, which could result in establishing new strategies for prevention and early detection of invasive breast tumors.

**Key Words:** Breast cancer - chromosomal aberrations - comparative genomic hybridization (CGH)

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### Introduction

Breast cancer is one of the most common cancers in women (Sloane et al., 1980; Branagan et al., 2002); however, only limited underlying karyotype abnormalities have been published in literature. Several recurrent clonal structural and numerical chromosomal abnormalities have been detected in primary breast cancer by cytogenetic analysis. Although the success rate and sensitivity of G-banding analysis have improved dramatically over the last few years, the frequency of aberrations may still be underestimated because of the inability to obtain analyzable metaphase cells from some tumors. Furthermore while karyotyping is ideal in revealing the tremendous genetic heterogeneity of breast cancer, the identification of all clonal genetic aberrations is sometimes difficult because of overwhelming complexity of changes (Dutrillaux et al., 1990; Pandis et al., 1995).

The molecular cytogenetic method of comparative genetic hybridization (CGH) may supply information on karyotype abnormalities (Du Manoir et al., 1993; Kallioniemi et al., 1994; Speicher et al., 1995; Waldmen

et al., 1996). CGH has been the technique of choice over the last 10 years for detecting DNA copy number differences. It is applicable to all uncultured tumors regardless of their mitotic activity or the complexity of chromosomal changes. While CGH does not detect all structural rearrangements, it offers an overview of DNA sequence copy number changes present in most of the tumor cells.

Many previous CGH studies of breast cancer were performed during the early phase of CGH technology development. The reliability and accuracy of CGH analysis has now improved dramatically as a result of recent developments in CGH technique and vigorous quality control (Karhu et al., 1997).

In the present study 16 invasive ductal breast carcinomas were analyzed with an optimized quality controlled CGH technique. It was aimed to define the most common genomic imbalances in a series of invasive breast cancers so as to correlate CGH findings with clinicopathological features, as well as to distinguish possible early genetic aberrations and changes which are important in classifying breast cancer.

<sup>1</sup>Dept. of Genomics, Cancer Institute Research Center, <sup>2</sup>Dept. of Medical Genetics, <sup>3</sup>Dept. of Reproductive Genetics, Reproductive Health Research Center, Tehran University of Medical Sciences, <sup>4</sup>Dept. of Cancer Research, Gene Institute, Tehran, <sup>5</sup>Dept. of Pathology, Tehran University of Medical Sciences, <sup>6</sup>Dept. of Surgical Oncology, <sup>7</sup>Dept. of Epidemiology, Cancer Research Center, Tehran University of Medical Sciences, Tehran, Iran \*For Corresponding author: P.O. Box 14197-83485 Tehran I.R. of Iran Fax: +9821-88887853 E-mail: saeed@ghaffari.org

**Table 1. Frequency and Percentage of 21 Chromosomal Aberrations Detected in 12 Samples of Invasive Ductal Breast Cancer by CGH**

Aberration	-8p	-16p-1p	-11q-22q	-13q	+20q	+8q	+17q	+1q	
Number	1	1	2	2	3	1	3	3	
Percent	4.8	4.8	4.8	9.5	9.5	14.3	4.8	14.3	14.3

## Materials and Methods

### Sample collection

Sixteen samples were randomly collected from patients diagnosed with invasive ductal carcinoma (confirmed by pathology), undergone modified mastectomy or breast conserving surgery between September 2003 and July 2004 in Cancer Institute. Sample tissues were stored at -80°C for a short period until DNA extraction.

### Genomic DNA preparation

Test and control high molecular weight genomic DNA samples were extracted from each sample by proteinase K and RNase digestions according to Sambrook et al., 2001. The control genomic DNA samples were extracted from chromosomally normal male (46, XY) and females (46, XX) blood samples.

### DNA sample labeling

Test and control human genomic DNA samples were respectively labeled indirectly with Dig- and Bio-Nick Translation Mixes (Roche Diagnostics, GmbH, and Mannheim, Germany). The control DNA samples were selected from the opposite sex (Male). This strategy is adopted as a mean of internal quality control and helps to insure the reliability of CGH results.

### Metaphase spreads

Metaphase spreads were prepared from phytohaemagglutinin (PHA) stimulated, peripheral blood lymphocytes of healthy males using standard procedures of hypotonic treatment and methanol/acetic acid fixation (3:1, v/v).

### Hybridization and post-hybridization washings

The following procedures were performed as described previously with few modifications included (Ghaffari et al., 1998). Each of metaphase chromosomes of normal

males went through a dehydration step at room temperature (RT) in 2 x SSC with pH of 7.0 (3 mol/l NaCl, 30 mmol/l Na<sub>3</sub>-citrate) and ethanol series (70%, 85%, 100%) for two minutes. Thereafter, slides were denatured for two minutes at 75°C on a slide warmer. Ten microliters of hybridization solution containing 1 µg of labeled test DNA, 1 µg of labeled control DNA, and 50 µg of unlabeled human Cot-1 DNA (Roche Diagnostics, GmbH, and Mannheim, Germany) was put on each slide, which then was covered by cover slip and sealed with rubber cement. After 72 hours of hybridization at 37°C in a humidified chamber, slides were washed for two minutes with 0.25 x SSC at 73°C, and thirty seconds with ST (2 x SSC, 0.05 tween 20) at RT, and then were stained with detection solution composed of avidin-FITC and antidigoxigenin-rhodamine for 30 minutes at RT and 1 x PBD for 3 x two minutes. Finally the samples were counterstained with 4, 6, diamino-2-phenylindol (DAPI, 0.1 µg/ml which resulted in coarse banding of the chromosomes, allowing individual chromosomes to be identified.

### Digital image acquisition, analysis and interpretation of the CGH results

Images for CGH analysis were obtained using a fully motorized epifluorescence microscope (DM6000 B, Leica Microsystems, Germany) equipped with a CCD camera (DFC350 FX monochrome digital camera, Leica Microsystems, Germany) and controlled by an image analyzing system (Leica CW4000, Cambridge, UK). For standard CGH analysis, green, red, and blue fluorescence images were taken from each high intensity, uniformly hybridized metaphase and analyzed as separate grey scale images. The image representing the blue DAPI counterstain was inverted and used for chromosome identification based on its coarse banding pattern. The mean of the individual ratio profiles of at least 10 and generally 20 metaphase spreads was calculated. The green and red fluorescence intensities were calculated and the green to red ratio profiles along the chromosome axis were displayed. For normalization of the ratio profiles, the model value of the green to red ratio for the entire metaphase was set to 1.0. Finally, the individual ratio profiles were combined to yield the average ratio profiles, which were displayed next to the chromosome diagrams with significant intervals of 0.8 and 1.2. Chromosomal

**Table 2. Demographic and Pathologic Data on Chromosomal Changes in 12 Invasive Ductal Carcinomas**

No	Age	Tumor Size (cm)	Side	P53	PR	ER	Her2	Histologic Grading	Nuclear Grading	Vascular Invasion	Neural Invasion	Component In situ	Lymph node Involvement	Chromosomal Aberration
2	54	2	L	+	+	+	+2	1	2	+	-	15%>	0/7	17q+
3	42	2.5	L	+	-	-	+3	2	2	+	-	-	3/16	13q-,1q+
5	71	3.8	R					2	2	+	-	15%>	6/7	20q+
8	53	3.5	L	-	+	+	+1	2	2	+	-	-	0/12	8q+
9	39	6	R	-	+	+	0	2	2	+	+	-	4/10	13q-
10	35	2	R	+	+	+	0	2	2	+	-	-	0/12	22q-,17q+
11	52	2.5	R	+	-	-	0	2	3	+	-	-	8/16	1q+,8q+, 11q-
12	49	4.5	R	+	+	+	0	2	2	+	+	25%>	4/6	1p-
13	70	8	L				0	2	2	+	+	5%>	8/34	13q-,17q+
14	65	2	L	-	-	+	+2	2	2	-	-	25%>	6/7	1q+,16q-,8p-
15	63	2.5	L	+	+	+	+2	2	2	+	-	-	0/8	1q+,11q-
16	37	4	L	+	+	+	+1	2	3	-	+	-	2/22	8q+,22q-

**Table 3. Demographic and Pathologic Data of 4 Samples of Invasive Ductal Breast Cancer with Normal CGH Results**

No	Age	Tumor Size cm	Side effects	Histologic Grading	Nuclear Grading	Vascular Invasion	Neural Invasion	In situ Component	Lymph node Invasion	ER	PR	P53	Her2	Chromosomal aberration
1	78	6	R	2	2	+	+		9/16	+	-	-	0	N
4	34	0.5	R	2	2	+	-	25%>	2/6	+	-	+	+1	N
6	42	1.5	L	2	2	+	+		0/8	+	+	-	0	N
7	38	1	L	3	2	+	-	25%<	0/14	+	+	-	+1	N

regions with a green to red ratio above 1.2 were considered to be overrepresented (gain), whereas regions with a ratio below 0.8 were considered to be under-represented (loss). These limit values were slightly different in each experiment depending on the thresholds deducted from the analysis of negative control experiments, in which two sets of differently labeled normal DNA samples were hybridized against each other.

## Results

Sixteen patients were enrolled in this study. The mean age of patients was 51.4 years old (SD=14.2 SE=3.5) with the range of (35-78) years. Seven cases had tumor in Right Breast (43.75%) and 9 cases had left side involvement (56.25%). The mean size of tumors was 3.27 cm (SD=2.03 SE=0.5) varying between (0.5-8) cm.

In histopathology studies, the nuclear grading was G2/3 in 14 cases and G3/3 in 2 cases according to the Richardson-Nottingham Modification, and the histopathology grading was G2/3 in 14 cases, G3/3 in 1 case, and G1/3 in one case. To sum up, from these 16 cases of invasive ductal carcinoma breast cancer, 12 samples had abnormal CGH results (75%), in whom 21 chromosomal imbalances were detected (Table 1).

The most prevalent chromosomal imbalances were chromosomal gains of +1q, +17q, and +8q and chromosomal loss of -13q. 33.35% of all breast cancer cases had gains of +1q, +8q or both and 3 had changes of (+1q, +8q or -13q) accounting for 47.65% of all tumor samples. More details are summarized in Table 2. Four samples had normal CGH results; the size of tumor varied between (0.5-6 cm) in this group and only two of them had axillary lymph nodes metastasis (Table 3).

In tumors with no metastasis to axillary lymph nodes (6 cases) six chromosomal imbalances were detected (mean of 1 chromosomal imbalance per tumor) whereas the number of chromosomal imbalances in tumors with lymph node metastasis (10 cases) was 15 (mean of 1.5 chromosomal imbalances per tumor). Chromosomal imbalances were +13q, +8q, +20q, +17q, -11q, -1p, -16q, and -22q in patients with positive axillary lymph node metastasis, and +8q, +1q, -22q, -11q in patients with no axillary lymph node involvement. All the three cases of -13q chromosomal imbalances were seen in axillary lymph node positive cases with metastatic breast tumor. Interestingly, the maximum number of chromosomal imbalances per tumor was 3 and was detected in two tumor samples both of which had axillary lymph node involvement.

More detailed data on distribution of tumor size, affected breast side, p53 and Her-2 presence, estrogen and

progesterone receptor presence, histologic grading, nuclear grading, vascular invasion, neural invasion and lymph node involvement are presented in Tables 2 and 3.

Due to the small sample size this study did not result in establishing any relationship between chromosome imbalances, histopathology and immunohistochemistry findings.

## Discussion

The importance of chromosomal abnormalities in the progression of breast cancer is reflected by the fact that most breast tumors studied by CGH showed a DNA copy number gain or loss. The frequency of chromosomal abnormalities in breast cancer previously reported ranges from 25%-81% (Thompson et al., 1993; Trent et al., 1993; pandis et al., 1995; steinarsdottir et al., 1995). In our study 75% of all samples assessed with CGH technique showed chromosomal abnormalities. Four samples had normal CGH results which may be either due to a true normal chromosomal constitution or minimal genetic changes which CGH technique is unable to detect.

21 chromosomal aberrations were detected in 16 breast cancer samples. The most prevalent aberrations were in 1q, 8q, 17q, 13q which is similar to other studies findings (Cingoz et al., 2003; Amiel et al., 2003; Micci et al., 2001; Rummukainen et al., 2001; Larramendy et al., 2000; Sinclair et al., 2003). Gains of 20q, 8q, 16q were prevalent in other studies, however, in this study was detected only in 1 case.

In this study chromosomal aberrations in -22q (in 2 cases) and -1p (in 1 case) which had not been reported in previous breast cancer studies, were also detected.

Comparing our results (cases diagnosed with invasive ductal carcinoma) with that of ductal carcinoma in situ in other studies, the most frequently changes in DCIS were gains at +1q, +5q, +8q and +17q as well as losses of -8p, -11q, -13q, -14q (Buerger et al., 1999, 2000; Deng et al., 1996; Moore et al., 1999; Reis-Filho et al., 2003; Simpson et al., 2005). While some chromosomal abnormalities are similar (+1q, +8q, -8q, -13q) there are some differences between the two groups.

Reviewing relevant studies revealed that some of the chromosomal aberrations reported in this research were found in other types of cancers previously. For instance, +17q in HCC (Kusano et al., 2002), +1q in HCC, endometrial cancer and fibroadenoma (Oga et al., 2002; Ojopi et al., 2001), +8q in most of solid tumors and astrocytoma (Tornillo et al., 2000; Nishizaki et al., 1998; Walch et al., 2000), +20q in colorectal cancers, esophageal squamous cell carcinoma, and head, neck and gastric carcinomas (Spicher et al., 1995; El Rifai et al., 1998;

Nishizaki et al., 2000; Maruno et al., 1999). It is also worth noting that the loss of 13q is reported in astrocytic tumors (Oga et al., 2002; Maruno et al., 1999), -16q in HCC, and fibroadenoma (Tornillo ; Ojopi et al., 2001; Walch et al., 2000; Ahmed et al., 2000), -8q in gastric adenocarcinoma, HCC, and choriocarcinoma (Tornillo et al., 2000; Spicher et al., 1995; Walch et al., 2000), and -22q in colorectal cancer (Nakao et al., 1998) .

In fact, the more research studies performed in this field in future, the more precise information would be obtained about the possible mechanisms of how oncogenes and tumor suppressors might interfere in causing these various abnormalities.

Gains of +1q, +8q are one of the most common genetic changes in breast cancer. In some other studies one or both of these changes were seen in 80% of of unselected breast cancers, and these 2 gains plus loss of -13q accounted for 91% of all tumors abnormalities (Trikkonen et al., 1998). In our study changes of (+1q, +8q, or -13q) account for 47.36% of all tumor abnormalities. To justify this difference between results it should be considered that selected invasive ductal carcinoma samples were studied in our research, whereas the samples in other studies were unselected breast carcinomas, nevertheless; more detailed studies are required to investigate each chromosomal aberration one by one in different tumoral cells for better understanding.

According to previous studies +20q is one of the prevalent chromosomal aberrations. It is seen in unspecific breast cancers and some other solid tumors (Amiel et al., 2003; Micci et al., 2001; Rummukainen et al., 2001; Larramendy et al., 2000), and its coincidence with estrogen positive tumors is more than estrogen negative ones (Cingoz et al., 2003), however, no similar correlation was found in invasive ductal carcinoma samples assessed in our study which may be the result of different tumor samples selected in each study.

The mean chromosomal aberrations per tumor in samples with metastasis to lymph nodes was more than lymph node negative ones (1.5 vs. 1). This finding undoubtedly confirms the fact that the average number of chromosomal aberrations increases with invasion and metastasis to lymph nodes (Albertson et al., 2003; Aubele et al., 2000; Buerger et al., 2000).

The pattern of aberrations at some chromosomal loci in unspecific breast cancer (+1q, +8q, +16q, +17q, +20q) differs significantly from carcinomas stratified by grade (Damiai et al., 1999; Selim AG et al., 2001); grade 1 (well differentiated) carcinomas frequently demonstrate gain of +1q and loss of -16q as well as an overall low incidence of alteration and amplification while higher grade (grade 2, 3), intermediate, and poorly differentiated carcinomas exhibit more genetic alterations, and more amplifications (+8q, +17q, +20q), and the frequency of -16q loss as compared to grade 1 carcinomas is significantly reduced (Damiani et al., 1999; Selim et al., 2001). Due to the small sample size, we were not able to find any correlation between any specific chromosomal aberration and tumor grading.

Our study, will pave the way to new studies for assessing the correlation between chromosomal

abnormalities and their relationship with clinical features of disease. By using new methods of cytogenetic assessment such as array CGH we can have a closer look at cancer cytogenetic assessment. Such approach will help us to use new technologies for early detection and management of breast cancer patients.

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