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# Prognostic and predictive value of 16p12.1 and 16q22.1 copy number changes in human breast cancer

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**Abstract** The present study investigated DNA copy number changes mapping to the p and q arms of chromosome 16 in breast cancer with the goal to determine their potential in identifying breast cancer patients with poor prognosis. We identified the minimal overlapping regions on chromosome 16 that are commonly deleted and amplified in breast tumors. Fluorescence in situ hybridization was used to screen a custom-made breast carcinoma tissue microarray representing all tumor grades, in order to detect DNA copy number changes mapping to 16p12.1 and 16q22.1. We generated 16q/16p ratios for each patient and examined the correlation between DNA copy number alterations and the patients' clinical and pathological parameters. We observed lower q/p ratios in grade I invasive carcinomas, compared with grade III carcinomas, which consistently showed high q/p ratios ($P < 0.0091$ and 0.0075). In addition, age adjusted for grade analysis revealed that tumors from younger patients (<45 yr) had significantly higher q/p ratios, suggesting that in younger individuals those tumors might be more aggressive ($P < 0.0001$). The finding that higher q/p ratios occur in younger patients offers a tool to identify high-risk individuals most likely to proceed to high grade. 

## 1. Introduction

Our goal with this study was to further characterize known breast cancer genomic amplifications and deletions, in order to provide new biomarkers for further investigation that could be used for the screening and prognosis of breast cancer patients. Multiple genes play key roles in breast cancer initiation and progression and provide robust tissue biomarkers that are used to determine the phenotype of breast cancer cells obtained by biopsy and to design therapy. The identification of cancer-causing loci relevant for diagnosis is challenging, given the high number of cytogenetic alterations observed, especially in high-grade tumors [1].

The Human Genome Project has been instrumental in the current advances toward dissecting the genome and determining which genes are responsible for human disease [2]. The Cancer Genome Anatomy Project (CGAP), through the Cancer Chromosome Aberration Project, advanced fluorescent in situ hybridization (FISH) mapping of DNA segments sequenced as part of the Human Genome Project. These served to make a cytogenetic link between DNA sequences involved in disease and their cytogenetic position [3]. Because of these efforts, we now can visualize and map genomic gains and losses in multiple tissue types and determine potential candidate genes mapping to those genomic regions. In the same effort to identify cancer-relevant DNA copy number changes, numerous studies performed on human primary tumors [4–7] and various mouse models for human cancer [8–11] have led to a fine mapping of chromosomal regions that undergo recurrent genomic alterations in multiple breast cancer subtypes, thus pinpointing novel candidate genes with biomarker potential.

The currently available biomarkers, such as estrogen receptor (ER), progesterone receptor (PR), and receptor tyrosine-protein kinase erbB-2 (HER2; encoded by the v-erb-b2 erythroblastic leukemia viral oncogene homolog 2 gene, *ERBB2*, alias *HER-2*), although essential for determining a regimen, do not enable a clear prediction of disease recurrence. The well-studied oncogenes and tumor suppressor genes do not necessarily represent the most effective diagnostic targets. Thus, there is a great need to

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identify molecular biomarkers that predict disease outcome and offer targets for novel therapies.

Cancer cells are characterized by gross aneuploidy and recurrent copy number alterations, with the final result being the deletion of tumor suppressor genes and oncogene amplification. In contrast to hematological malignancies, in which chromosomal translocations resulting in gene fusions and consequent oncogene activation are a frequent event, tumors of epithelial origin more often exhibit cells with a high degree of aneuploidy and recurrent chromosome gains and losses.

Numerous studies have been performed genome-wide to identify and map regions of recurrent DNA copy number variation in breast cancer patients (for a review, see Albertson, 2003 [12]). The regions of genomic imbalances frequently map to chromosomes 8p and 8q, 11q, 13q, 16p and 16q, 17p and 17q, and 20q [13−15]. These genome-wide analyses are essential for pinpointing chromosomal regions frequently altered in breast cancer, but they fail to deliver a tool that can be used in the setting of a cytogenetic laboratory to help in predicting clinical outcome. Copy number changes mapping to chromosome 16 are of particular interest because they have been reported as the most frequent alteration observed in multiple studies. In particular, loss of 16q has been associated with better prognosis [16−18]. Most notably it is observed in ductal carcinoma in situ (DCIS), indicating that 16q loss occurs early during tumorigenesis, and it is present in otherwise karyotypically normal breast cancer cells. All these observations suggest that copy number changes mapping to chromosome 16 are an early initiating event in breast tumorigenesis [19] and that gain and losses of 16q and 16p play a central role in tumor development and clinical outcome.

The present study was based on the analysis of DNA copy number changes of two bacterial artificial chromosome (BAC) clones mapping to chromosome 16p and 16q, selected based on their differential copy number changes in lobular versus ductal breast tumors [1] and in stage I versus stage III ductal breast tumors [20]. Although aberrations of whole chromosome 16 arms have been reported, studies suggest the presence of hot spot aberrations with specific minimal overlapping regions [1,20]. The clones we selected map to those high-frequency and recurrent changes on the p and q arms of chromosome 16. Because with an interphase FISH mapping approach using locus-specific probes for one single chromosome we cannot account for gross aneuploidy, we selected one probe for the p arm and one of the q arm of chromosome 16 and expressed the results as q/p ratios.

## 2. Materials and methods

### 2.1. Patient cohort and tissue microarray construction

Tissue microarrays were generated in our pathology department (M.H.O.). Patients were selected based on pathological analysis of tissue diagnosed as intraductal breast cancer of any grade. After selection, the patient was assigned a code: A1−A10, B1−B9, C1−C9, D1−D9, and E1−E9, with the code annotated as invasive, in situ (DCIS), or benign breast. Each tumor was formalin fixed and paraffin embedded according to our institutional pathology department protocol. Before the study, morphology was examined using a routine hematoxylin−eosin stain to find areas corresponding either to the part of the tumor with the highest density of cells or to normal breast tissue surrounding the tumor. Tissue microarrays were constructed with three 1-mm cores per case containing representative tissue, using standard technique. The tumors were sectioned into 4-μm-thick sections, which were fixed to a glass microscope slide.

The samples used in this study were isolated from paraffin blocks generated from leftover biopsies and thus they represent exempt research that does not require patient consent. They are part of a collection of breast tissues and samples under an approved protocol (CCI protocol no. 2007-433, approved by the Albert Einstein College of Medicine Committee on Clinical Investigations).

### 2.2. Selection of BAC clones

After a systematic review of the literature [1,18,20] we selected BAC clones in the minimal overlapping region of amplification using chromosome map information from online databases of the Cancer Chromosome Aberration Project (http://cgap.nci.nih.gov/Chromosomes/CCAP_BAC_Clones) and the University of California, Santa Cruz, Genome Bioinformatics website (http://www.genome.ucsc.edu/). The BAC clones RP11-5A19 and RP11-21M24, mapping respectively to 16q22.1 and 16p12.1, were obtained from the BACPAC Resources Center at Oakland, CA (http://bacpac.chori.org/). Each clone is mapped to the hg18 build of the human genome (March 2006) and is annotated with known genes through the National Center for Biotechnology Information database (NCBI map viewer) (http://www.ncbi.nlm.nih.gov/).

### 2.3. Isolation of BAC clone DNA and probe labeling

The BAC clones were grown overnight with shaking at 37°C in 250-mL vented sterile flasks in Luria broth (Fisher Scientific, Pittsburgh, PA) with 25 μg/mL chloramphenicol (MP Biomedicals, Solon, OH). Sterile picks were used to inoculate the Luria broth with a single BAC grown on agar. The DNA was isolated using a maxi-prep protocol involving three steps of treatment: Tris 50−10 mmol/L with 100 μg/mL RNase A EDTA (Sigma-Aldrich, St. Louis, MO); 200 mmol/L sodium hydroxide in 1% SDS (Sigma-Aldrich); 3 mol/L potassium acetate pH 5.5 (Sigma-Aldrich). The pellet containing DNA was resuspended in 150 μL water containing 5 μL RNase stock at room temperature with shaking overnight. The DNA was