Genomic and Expression Analysis of the 8p11–12 Amplicon in Human Breast Cancer Cell Lines

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ABSTRACT

Gene amplification is an important mechanism of oncogene activation in breast and other cancers. Characterization of amplified regions of the genome in breast cancer has led to the identification of important oncogenes including erbB-2/HER-2, C-MYC, and fibroblast growth factor receptor (FGFR) 2. Chromosome 8p11-p12 is amplified in 10–15% of human breast cancers. The putative oncogene FGFR1 localizes to this region; however, we show evidence that FGFR inhibition fails to slow growth of three breast cancer cell lines with 8p11-p12 amplification. We present a detailed analysis of this amplicon in three human breast cancer cell lines using comparative genomic hybridization, traditional Southern and Northern analysis, and chromosome 8 cDNA microarray expression profiling. This study has identified new candidate oncogenes within the 8p11-p12 region, supporting the hypothesis that genes other than FGFR1 may contribute to oncogenesis in breast cancers with proximal 8p amplification.

INTRODUCTION

In a significant subset of human breast cancers (HBC), as in other solid tumors, gene amplification and overexpression is an important mechanism for oncogene activation. In breast cancer, a number of discrete amplicons have been identified including 17q12 (erbB-2/HER-2), 8q24 (C-MYC), 11q13 (CCND1/PRAD1), and 10q26 [fibroblast growth factor receptor (FGFR) 2]. The 17q12 amplicon is well characterized, and overexpression of erbB-2 plays an important causal role in breast cancer development (1–3). However, other genes within the 17q12 amplicon, such as Grb7, are also overexpressed (4) and may play a role in breast cancer progression, possibly interacting with erbB-2 in mediating transformed phenotypes (5).

The 10q26 amplicon includes FGFR2, which is amplified and overexpressed in 4–12% of breast cancers (6, 7) as well as the SUM-52 cell line developed in our laboratory (8). We have shown that expression of FGFR2 in human mammary epithelial (HME) cells is transforming.5

Amplification of 8p11-p12, including the FGFR1 region, has been reported in 10–15% of breast cancers (6, 9, 10), and may be associated with a poor prognosis, particularly for node-positive patients (11). Amplification of the 8p11–12 region has been associated with estrogen receptor-positive tumors and lobular histology (12). FGFR1 amplification is associated with overexpression in a subset of those tumors (10), but in the others, FGFR1 expression is comparable with levels in normal breast tissue (9). Furthermore, FGFR1 is excluded from the amplicon in some tumors (13).

In our panel of 11 breast cancer cell lines, 3 lines (SUM-44, SUM-52, and SUM-225) have an amplification in the 8p11-p12 region (8, 14, 15). However, unlike FGFR2, we have been unable to find direct evidence that FGFR1 is the driving oncogene in these cell lines. We have undertaken a detailed genomic and expression analysis of the 8p11-p12 amplicon in these three cell lines, using array comparative genomic hybridization (CGH), Southern blot analysis, Northern blot analysis, and chromosome 8-specific cDNA microarrays. These experiments have identified new candidate oncogenes and have provided support for the hypothesis that genes other than FGFR1 may play a causal role in breast cancer progression in tumors with amplification in the 8p11-12 region.

MATERIALS AND METHODS

Bacterial Artificial Chromosome (BAC) Array CGH. Array CGH was carried out using arrays of 2464 BAC clones each printed in triplicate (Human-Array1.14) according to published protocols (16, 17). Briefly, cell line and normal male reference DNA (300 ng each) were labeled by random priming in separate 50-μl reactions to incorporate Cy3 and Cy5, respectively. The labeled DNAs were combined with 100 μg human Cot-1 DNA and hybridized to the BAC arrays for ~48 h at 37°C. After posthybridization washes, the arrays were mounted in a solution containing 90% glycerol, 10% PBS, and 1 μM 4′,6-diamidino-2-phenylindole, and sealed with a coverslip. A custom built CCD camera system was used to acquire 16 bit 1024 × 1024 pixel 4′,6-diamidino-2-phenylindole, Cy3 and Cy5 images (18). Image analysis was carried out using University of California San Francisco SPOT software (19). The log2ratio of the total integrated Cy3 and Cy5 intensities for each spot after background subtraction was calculated, normalized to the median log2 ratio of all of the clones on the array, and the average of the triplicates calculated using a second custom program, SPROC. Automatic data filtering to reject data points based on low 4′,6-diamidino-2-phenylindole intensity, low correlation between Cy3 and Cy5 within each segmented spot, and low reference/4′,6-diamidino-2-phenylindole signal intensity was also carried out using SPROC. Data files were subsequently manually edited by rejecting clones for which only one spot of the triplicate survived after SPROC analysis and for which the SD of the log2 ratio of the triplicate was >0.2. For each tumor, the data are plotted as the mean log2 ratio of the triplicate spots for each clone normalized to the genome median log2 ratio. The clones are ordered in position in the genome according to the University of California-Santa Cruz Biotechnology (UCSC) Human Genome Working Draft.6

Cell Lines and FGFR Inhibitor Growth Experiments. The isolation and culture of the SUM-44, SUM-52, and SUM-225 HBC cell lines have been described in detail previously (14, 20, 21). SUM-44 cells were grown under serum-free conditions using a base medium of Ham’s F-12 supplemented with 0.1% BSA, insulin (5 μg/ml), hydrocortisone (1 μg/ml), 5 mM ethanolamine, 10 mM HEPES, transferrin (5 μg/ml), triiodothyronine (10 μg/ml), 50 μM sodium selenite, gentamicin (5 μg/ml), and fungizone (0.5 μg/ml). SUM-52 and SUM-225 cells were routinely cultured in Ham’s F-12 supplemented with 5% fetal bovine serum, insulin (5 μg/ml), hydrocortisone (1 μg/ml), gentamicin (5 μg/ml), and fungizone (0.5 μg/ml). Detailed information regarding the SUM series of HBC cell lines is accessible online.7 The MCF10A cell line, a spontaneously immortalized normal mammary epithelial cell line (22), was cultured in the same serum-free medium supplemented with epidermal growth factor (10 ng/ml).

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Note: M. E. Ray and Z. Q. Yang contributed equally to this work.

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6 Internet address: http://genome.ucsc.edu.

7 Internet address: http://www.cancer.med.umich.edu/breast_cell/prodution/index.html.
For the FGFR inhibitor experiments, cells were grown in their normal growth medium supplemented with the inhibitor PD 173074 (Ref. 23; dissolved in DMSO) at a final concentration of 1 μM. PD173074 was provided by Dr. Wilbur Leopold and Pfizer Pharmaceuticals (Ann Arbor, MI). Growth media for control cells were supplemented with equivalent volumes of DMSO with no inhibitor. Cells were counted at the indicated time points using a Coulter Counter (Beckman Coulter, Miami, Florida) per the manufacturer’s instructions.

Southern and Northern Analyses. Known genes and expressed sequence tags (ESTs) from the 8p11–12 region were selected from the databases of the UCSC and the National Center for Biotechnology Information. cDNA clones of these genes and ESTs were purchased from the ResGen Invitrogen Corporation. Plasmid DNA was isolated using a standard plasmid isolation protocol (QiAprep; Qiagen), and digested with the appropriate restriction endonuclease. Digested DNA was electrophoresed through an agarose gel, and checked for consistent banding patterns and/or directly sequenced to verify the clone identity. The insert DNA was extracted and purified (QiAqick gel extraction kit; Qiagen), and prepared as probes for Southern blot and Northern blot hybridization.

Cellular DNA and total RNA was prepared from cultured HBC cell lines and the HME cell line (MCF10A) by standard methods. For Southern blot analysis, 10-μg aliquots of EcoRI-digested DNA were electrophoresed on 0.8% agarose gels and transferred to nylon membranes (Hybond). For Northern blot experiments, 20-μg aliquots of total RNA were electrophoresed in 1.0% agarose/0.67 M formaldehyde gels and transferred to positively charged nylon membranes (Hybond). Each membrane was hybridized with [α-32P]dCTP-labeled cDNA probes, which were labeled using the Mega-prime DNA labeling system (Amersham Pharmacia Biotech). Hybridizations were performed using ExpressHyb hybridization solution (Clontech), according to the manufacturer’s instructions. Blots were exposed for autoradiography for 8–36 h at −80°C as necessary to achieve optimal exposure before developing.

Expression Profiling of Human Chromosome 8 Using a Custom cDNA Microarray. A custom chromosome 8 cDNA array was produced at the University of Michigan Comprehensive Cancer cDNA and Affymetrix Microarray Core (24). This array consisted of PCR products amplified from 677 Research Genetics (Huntsville, AL) human cDNA clones of which the sequences mapped to chromosome 8 according to information from the National Center for Biotechnology Information Ensemble database (25), and the UCSC Genome Bioinformatics database (26). A complete list of cDNAs spotted onto the chromosome 8 array can be obtained on the internet (27).

Total RNA was prepared from SUM-44, SUM-52, and SUM-225 breast cancer cell lines, and from MCF10A HME cells using TRIzol reagent (Invitrogen Life Technologies, Inc., Carlsbad, CA) following the manufacturer’s instructions. The RNA was additionally purified using RNeasy reagents (Qiagen, Valencia, CA). RNA quality was confirmed by denaturing agarose gel electrophoresis. Twenty μg total RNA was labeled with Cy3 or Cy5 reagent using the CyScrite First-Strand cDNA Labeling Kit and purified on AutoSeq G50 columns (Amersham Biosciences, Piscataway, NJ) following the manufacturer’s instructions. Cy3- and Cy5-labeled samples were pooled and purified to a volume of ~5 μL. Microarray slides were prehybridized for 45 min in 5× SSC, 0.1% SDS, 25% formamide, and 1% BSA at 42°C. Slides were then rinsed with ddH2O, dried, and preheated to 68°C. SlideHyb #1 hybridization buffer (75 μL; Ambion, Houston, TX) was preheated to 68°C and added to the pooled labeled cDNAs. The probe was incubated at 68°C and added to the microarray surface under a lifterslip (Erie Scientific, Portsmouth, NH). The slides were hybridized in a dark, humidified chamber at 42°C for 10 min. The slides were then briefly rinsed in water, dried, and stored in the dark until scanning with a GenePix 4000A Axon laser scanner and associated software (Axon Instruments, Burlingame, CA). Photomultiplier tube settings were manually adjusted to achieve red (Cy5) to green (Cy3) normalization factors close to 1. Each experiment was performed in duplicate using a color switch. Microsoft Excel spreadsheets were generated for data analysis. Chromosome 8 clones were ordered using the April 2003 freeze of the UCSC Human Genome Working Draft. Array elements with signal sum of medians ordered using the April 2003 freeze of the UCSC Human Genome Working Draft. Array elements with signal sum of medians ordered using the April 2003 freeze of the UCSC Human Genome Working Draft.
proliferation of SUM-44 or SUM-225 cells (Fig. 2B). In contrast, the FGFR inhibitor did slow proliferation of SUM-52 cells, which overexpress FGFR2 at the message and protein level (8). These results suggest that whereas FGFR2 amplification and overexpression can lead to a transformed phenotype, FGFR1 does not appear to be a driving oncogene in any of the SUM breast cancer cell lines containing the 8p11-12 amplicon.

Detailed Mapping of the 8p11-12 Amplicon. Because we were unable to find direct evidence implicating FGFR1 as the target oncogene for the 8p11-12 amplicon, we sought to define the amplification and expression status of other genes on the 8p11-12 amplicon. To map the amplified region in these three cell lines in detail, and to define the minimal common regions of amplification, the copy number for known genes and ESTs distributed across bands 8p11-p12 was determined by Southern blot analysis. Representative examples are shown in Fig. 3.

A total of 33 known genes or ESTs were found to be amplified in at least one of the three cell lines. Of these, 11 known genes were amplified in only one of the three cell lines (NRG, MGC1136, ASH2L, STAR, ADAM18, SFRP1, ZNF220, IKK-β, POLB, VDAC3, and SLC20A2), whereas 15 known genes or ESTs were amplified in two of the three cell lines (FLJ14299, PROSC, ADRB3, LSM1, BAG4, KIAA0725, WHSC1L1, FGFR1, FLJ25409, ADAM2, Hs.170537, FLJ13842, ANK1, BRF2, and EIF4EBP1). Only 5 known genes (HTPAP, TACC1, INDO, TC-1, and RCP) and two ESTs (Hs.156542 and Hs.188833) were amplified in all three of the cell lines.

There was significant heterogeneity in the copy number gains detected by Southern analysis, ranging from dramatic, high level amplification such as HTPAP in SUM-44 cells, to subtle low level copy number gain such as HTPAP in SUM-225 cells.

On the basis of the Southern blot studies in the three cell lines, the smallest common region of gene amplification in the three cell lines does not include FGFR1 (Table 1), supporting the hypothesis that genes other than FGFR1 from the 8p11-12 amplicon may play important roles in breast cancer development.

To confirm that the amplicon structure as detected in the three breast cancer cell lines is consistent with 8p11-12 amplifications that occur in primary breast cancers, quantitative PCR analysis was carried out using genomic DNA obtained from microdissected breast cancer specimens derived from our frozen breast cancer bank. The PCR experiments were performed using primers specific for FLJ14299, LSM1, FGFR1, and TC-1, as these genes span the amplicon detected in the cell lines. Of the 32 breast cancers examined, 8 showed evidence of high level amplification (>4-fold) in at least part of the 8p11-12 region. Interestingly, TC-1 and FLJ14299 were most commonly amplified, whereas FGFR1 was only found to be >4-fold amplified in 1 of 32 primary breast cancers (Table 2). These results are consistent with those obtained from the cell lines, which suggest that regions of 8p11-12 flanking the FGFR1 locus may be of greater significance in breast cancer. These results also suggest that genes flanking the FGFR1 locus may be amplified in a higher proportion of breast cancers than has been published previously.

Gene Expression Analyses of the 8p11-12 Amplicon: Chromosome 8 cDNA Microarray Expression Profiling, Northern Analysis, and Quantitative RT-PCR. To rapidly determine whether genes from 8p11-12 were overexpressed in association with amplification, we performed expression profiling using a custom human chromosome 8 cDNA microarray. The microarray was designed by querying...
the human chromosome 8 Unigene database (National Center for Biotechnology Information) to identify clones from a sequence-verified Research Genetics cDNA library, which localized to chromosome 8. These clones were assembled into a sublibrary, and cDNA microarrays were constructed. The microarray contains a total of 1134 elements, with 939 clones localizing to chromosome 8 and 116 clones localizing to 8p11–12.

Expression profiling of breast cancer cell lines SUM-44, SUM-52, and SUM-225 versus the HME cell line MCF10A as control revealed that several genes from the amplicon region were overexpressed in the SUM cell lines (Fig. 4). In particular, cDNAs for FLJ14299, PROSC, RCP, EIF4EBP1, LSM1, HTPAP FGFR1, and LOC51125 were up-regulated >3-fold in SUM-44 cells. SUM-225 cells also showed significant up-regulation of FLJ14299 and RCP. SUM-52 cells showed more moderate up-regulation of RCP and EIF4EBP1, but showed more significant up-regulation of C8ORF4 (TC-1) and LOC51125.

To validate the expression profiling data and to extend our gene expression analysis to include genes not represented on the cDNA microarray, we also performed Northern hybridizations comparing the SUM HBC cell lines and HME cell controls. Representative results of Northern blot hybridizations are shown in Fig. 5, and the gene overexpression results are summarized in Table 1.

The Northern results demonstrated a high level of qualitative agreement with cDNA microarray results; however, the microarray data appeared to quantitatively underestimate the fold-differences in gene expression observed by Northern analysis. In a few instances, Northern hybridization detected gene expression patterns inconsistent with the cDNA microarray analysis. This was true in particular for TACC1 expression, which was found to be overexpressed by Northern (Fig. 5) as well as Western (data not shown) analysis. We note that many of the TACC1 cDNA clones on the cDNA microarray were from untranslated portions of the gene, whereas the Northern probe was the full-length coding sequence. We postulate that alternative mRNA splicing within certain cell types may occasionally limit the sensitivity of microarrays composed of cDNA fragments from spliced portions of certain genes.

Several genes from the amplicon show a close correlation between DNA amplification and mRNA expression (Figs. 3–5). This correlation is noticeable for FLJ14299, LSM1, RCP, and HTPAP. FLJ14299 and RCP showed particularly high levels of overexpression in SUM-44 and SUM-225 cells (Figs. 4 and 5). Interestingly, C8ORF4 (TC-1) was found to be overexpressed only in SUM-52 cells although it is amplified in all three of the cell lines (Figs. 3–5). Two genes, HTPAP and TACC1, show amplification and overexpression in all three of the cell lines with the amplicon, making them particularly interesting candidate genes that warrant further study for their roles in HBC.
DISCUSSION

Aberrations of chromosome 8 have been observed in many human cancers, and several studies have shown that chromosome 8 is a frequent target for gene amplification and gene loss in breast cancer. In particular, copy number increases are commonly observed in distal 8q (12, 25–29) and in the 8p11-p12 region (6, 9). The 8p region is also commonly associated with copy number decreases in breast cancer (30–33). Indeed, gene amplification at 8p11-p12 often occurs in a background of gene loss, and that is the case with the cell lines used in this study (14). Our results, derived from chromosome CGH and BAC array CGH analysis of the 11 breast cancer cell lines in our panel, are consistent with the idea that 8p11-p12 is a common region of amplification, and could, therefore, harbor important breast cancer oncogenes.

The FGFR1 gene has long been considered to be the best candidate oncogene at this locus. However, the exact involvement of this receptor in the progression of breast cancer is unclear, because it is not consistently present in the amplification unit and is not always overexpressed when amplified. The results obtained with our cell lines are not consistent with a dominant, transforming role for FGFR1. Among our three cell lines with the ampiclon, FGFR1 is overexpressed at the message level in only one, and even in this line, the FGFR1 receptor is not overexpressed at the protein level. Thus, it was not surprising that an FGFR-specific tyrosine kinase inhibitor did not influence the growth of these cells.

TACC1 is another gene from the region that has been implicated in breast cancer development (34). The TACC gene family encodes centrosomal proteins that may play roles in microtubule regulation and spindle function, and, thus, may be an important driver of genomic instability in cancer cells (35–39). Still et al. (34) demonstrated that TACC1 is overexpressed in some breast cancers, and it is transforming in NIH3T3 cells. The data from our work are consistent with a possible transforming role for TACC1, because it is the only transcribed gene present within the smallest common region of gene amplification in the three SUM lines with the ampiclon. Furthermore, TACC1 is overexpressed at the mRNA level in these cells. However, other reports suggest that the TACC proteins are lost or down-regulated in breast cancers and propose that they might be tumor suppressor genes, which play a role in mRNA homeostasis (40, 41). Interpretation of this result is complicated by the fact that most of 8p is lost in a high proportion of breast cancers making definitive identification of the tumor suppressor gene at this locus difficult. Clearly, additional work will be required to precisely define the transforming function, if any, of TACC1 in HBC.

Several other genes are present within the smallest common amplification unit of the three SUM lines, providing circumstantial evidence for roles for these genes in carcinogenesis. However, in addition to the small common region of amplification, each cell line also has unique regions of gene amplification containing several interesting and highly transcribed genes. Thus, it remains possible that the 8p11–12 region, like other “hot spots” for gene amplification in breast cancer, contains multiple genes, which contribute to cell transformation. For this reason, we have carefully defined the amplification and expression pattern of genes in the 8p11–12 region in three cells lines with the ampiclon and have identified several candidate genes that need to be studied further.

In addition, we found that in the SUM-225 cells, the region of gene amplification may extend across the chromosome 8 centromere into the 8q11 region. The peri centromeric region is transcript poor, and currently there are few candidate genes from this region. However, this region is worthy of further study because, among all of the amplified regions of chromosome 8 in these cell lines, the 8q11/BAC clone CGH peak in SUM-225 showed the highest copy number increase.

Several known genes and ESTs from the ampiclon were found to be overexpressed in one, two, or all three of the cell lines. The known genes include LSM1, TC-1, RCP, FLJ14299, EIF4EBP1, and HTPAP. LSM1, also termed cancer-associated Sm-like protein, was originally cloned from human pancreatic cancers as an up-regulated gene (42). Antisense LSM1 RNA is able to alter the transformed phenotype of pancreatic cancer cells by reducing their ability to form large colonies.

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4 EST, expressed sequence tag; UCSC, University of California-Santa Cruz Biotechnology Center; BAC, bacterial artificial chromosome; FISH, fluorescence in situ hybridization; ND, not determined.

Table 2. Greater than 4-fold copy number increase assessed by quantitative genomic PCR analysis of microdissected primary breast cancer specimens

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<tr>
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## 8p11–12 AMPLICON ANALYSIS IN BREAST CANCER CELLS

![Diagram](image)

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Fig. 4. Chromosome 8 cDNA microarray gene expression profile of the amplified regions of 8p11–12 in SUM-44, -52, and -225 cells versus MCF10A HME control cells. The gene symbol, clone identification number, chromosome band, chromosome map location based on the April 2003 freeze of the UCSC Genome Working Draft and the expression ratios for the three cell lines are displayed. Values >2.0 are highlighted in red, whereas values <0.5 are in green. Defective data points or those with a sum of median signals <1000 are omitted.
in soft agar when compared with untransfected cells and can decrease pancreatic tumor growth in vivo (43).

TC-1 is a novel gene that was originally cloned from suppression subtractive hybridization between a papillary thyroid carcinoma and its surrounding normal thyroid tissue (44). Overexpression of TC-1 in thyroid cancer was found in 15 of 16 paired primary samples. In addition, serial analysis of gene expression of normal mammary epithelial cells, and in situ, invasive, and metastatic breast carcinomas demonstrated high levels of TC-1 expression in intermediate-grade/estrogen receptor-positive breast cancers (45). RCP has been shown to interact with Rab4 and Rab11, small GTPases belonging to the Ras superfamily, and may function in regulation of the membrane trafficking of endosomal receptor recycling (46, 47).

FLJ14299 is a novel gene that contains a C2H2 motif. C2H2 zinc finger domains are nucleic acid-binding protein structures that are also present in several tumor-related genes such as BCL6 (48), ZNF217 (49), and GLI (50). FLJ14299 is of particular interest, because it is expressed at moderate levels in MCF-10A normal HME cells and is overexpressed to very high levels in the breast cancer cells with the amplicon.

HTPAP is a novel gene containing a type 2 phosphatidic acid phosphatase domain. Finally, the product of EIF4EBP1 is localized randomly to the mTOR pathway, participating in the regulation of the formation of eIF4F complex and acting to negatively regulate translational initiation (51). As indicated above, EIF4EBP1 is localized randomly to chromosome 8 in the most recent genome working draft. Given that EIF4EBP1 colocalizes to the same BAC clone with ADRB3, we postulate that it will eventually map close to that gene.

These candidate genes are not only amplified and overexpressed in the SUM HBC cell lines. Expression profiling studies of high- and low-risk patient groups shows that several of the candidate genes are also overexpressed among primary breast cancer specimens (52). Of 34 high-risk breast cancer patients who developed metastatic disease in <5 years, 3 patients showed >2-fold up-regulation of multiple genes within the amplicon region including PROSC, EIF4EBP1, ASH2L, LSM1, BAG4, KIAA0725, WHSC1L1, FGFR1, FLJ13842, SFRP1, LOC51125, and ZNF220. Among 44 lower-risk patients with >5 years to development of metastatic disease, 4 patients showed up-regulation of amplicon genes including PROSC, EIF4EBP1, ASH2L, LSM1, BAG4, KIAA0725, WHSC1L1, FGFR1, TACC1, C8ORF4(TC-1), FLJ13842, and SFRP1. One of 19 additional sporadic breast cancer specimens also showed up-regulation of PROSC, EIF4EBP1, ASH2L, LSM1, BAG4, KIAA0725, and WHSC1L1. These data are consistent with previously published 10–15% prevalence of the 8p11–12 amplicon in HBC (6, 9, 10) and indicate that the expression profiles of these cell lines are highly consistent with primary HBCs that harbor the amplicon.

Our results show that the 8p11–12 amplicon resembles other amplicons that have been identified in breast cancer, such as those found on 17q, 11q13, and 20q13, in several ways. Each of these amplicons occurs nonrandomly in a significant fraction of breast cancer and each region harbors several genes that have been implicated in breast cancer development. The observation that all of the well-defined breast cancer amplicons contain several candidate genes suggests that these regions may be “hot spots” for amplicon formation precisely because they may contain multiple genes with oncogenic potential. This view is in contrast to the assumption held previously that each amplicon contains only a single dominant oncogene. This distinction is important because past work has relied on the identification of commonly amplified and overexpressed genes within large panels of cancers to provide evidence for transforming function, implicating genes as candidate oncogenes. However, if amplicons, by their very nature, contain more than one functional oncogene, then identifying oncogenes on the basis of common amplification and overexpression patterns would be likely to miss, or even rule out, genes that actually have functional significance in cancer progression.

For this reason, we used an alternate approach by using three well-characterized cell lines developed in our laboratory, and identified candidate oncogenes based on amplification and expression patterns in each of the cell lines. These candidate genes can now be tested for transforming function either by overexpressing the genes in immortalized HME cells, or by disrupting their function in the breast cancer cells themselves using antisense- or RNA-interference-based approaches. This direct, functional assessment of the transforming potential of these candidate oncogenes will suggest whether these genes represent good targets for novel therapeutics.

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47
Genomic and Expression Analysis of the 8p11–12 Amplicon in Human Breast Cancer Cell Lines

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