Characterization of the Recurrent 8p11-12 Amplicon Identifies PPAPDC1B, a Phosphatase Protein, as a New Therapeutic Target in Breast Cancer

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Abstract

The 8p11-12 chromosome region is one of the regions most frequently amplified in breast carcinoma (10–15% of cases). Several genes within this region have been identified as candidate oncogenes, as they are both amplified and overexpressed. However, very few studies have explored the role of these genes in cell transformation, with the aim of identifying valuable therapeutic targets. An analysis of comparative genomic hybridization array and expression profiling data for a series of 152 ductal breast carcinomas and 21 cell lines identified five genes (*LSM1, BAG4, DDHD2, PPAPDC1B*, and *WHSC1L1*) within the amplified region as consistently overexpressed due to an increased gene copy number. The use of small interfering RNA to knock down the expression of each of these genes showed the major role played by two genes, *PPAPDC1B* and *WHSC1L1*, in regulating the survival and transformation of two different cell lines harboring the 8p amplicon. The role of these two genes in cell survival and cell transformation was also confirmed by long-term knockdown expression studies using short hairpin RNAs. The potential of *PPAPDC1B*, which encodes a transmembrane phosphatase, as a therapeutic target was further shown by the strong inhibition of growth of breast tumor xenografts displaying 8p11-12 amplification induced by the silencing of *PPAPDC1B*. The oncogenic properties of *PPAPDC1B* were further shown by its ability to transform NIH-3T3 fibroblasts, inducing their anchorage-independent growth. Finally, microarray experiments on *PPAPDC1B* knockdown indicated that this gene interfered with multiple cell signaling pathways, including the Janus-activated kinase-signal transducer and activator of transcription, mitogen-activated protein kinase, and protein kinase C pathways. *PPAPDC1B* may also potentiate the estrogen receptor pathway by down-regulating *DUSP22*. [Cancer Res 2008;68(17):7165–75]

Introduction

Recurrent chromosomal alterations are a hallmark of cancer cells and represent critical events in tumor development. In particular, oncogene activation through increased gene copy number resulting in overexpression contributes to the malignant transformation of various human solid cancers, including breast cancer (1–3). In breast cancer, major recurrent amplicons include 17q12 (*ERBB2/HER-2*), 8q24 (*MYC*), 11q13 (*CCND1*), 20q13, and 8p11-12 (4). The 17q12 amplicon, which has been extensively characterized, provides a clear illustration of the potential of tumor genomics for identifying drug targets. Indeed, this amplification consistently leads to overexpression of the *ERBB2* receptor, which can be efficiently targeted by specific inhibitors, such as Herceptin, now used to treat *ERBB2*-positive breast carcinoma (5). Two other genes within the 17q12 amplicon, *GRB7* and *STARD3*, have also recently been shown to play a causal role in tumor development (6). Major efforts are now under way to characterize potential drug targets in other breast cancer amplicons (6–8).

The 8p11-12 amplicon is observed in approximately 10% to 15% of breast carcinomas and is correlated with histologic grade, Ki-67 proliferation index, and poor prognosis (9–15). Recent studies have improved the definition of this amplicon and have identified several candidate oncogenes based on significant correlations between gene amplification and gene overexpression (13–17). However, no clear consensus about the minimal region of amplification or about the driver genes of the amplicon has emerged from these expression/amplification correlation studies. This lack of consensus highlights the need for functional experiments to validate the oncogenic properties of the various candidate oncogenes. The functional role of several putative oncogenes has already been explored. The inhibition of *FGFR1*, a candidate driver gene, does not slow the proliferation of all breast cancer cell lines with 8p11-12 amplification (16, 18), whereas *LSM1* has been shown to display transforming activity in all human breast cancer cell lines tested harboring the 8p amplicon (19). The first systematic functional study of the genes overexpressed as a consequence of the 8p11-12 amplification in breast tumors was recently published (15). Based on their ability to transform the immortalized human mammary epithelial cell line MCF10A, rendering it independent of specific growth factors *in vitro*, three genes were identified as breast cancer oncogenes: *BAG4, LSM1*, and *C8orf4*.

Using a very different functional approach, based on the small interfering RNA (siRNA)-mediated knockdown of gene expression, we also aimed to identify putative oncogenes and therapeutic targets among genes amplified and overexpressed in breast tumors

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with 8p amplification. We report the analysis, by array comparative genomic hybridization (CGH) and gene expression profiling, of a series of 152 ductal breast carcinomas and 21 cell lines. This analysis identified five genes (*LSM1, BAG4, DDHD2, PPAPDC1B*, and *WHSC1L1*) within the amplified region as consistently overexpressed due to an increase in gene copy number. Knocking down the expression of each gene, using siRNA, showed the major oncogenic role of two genes, *PPAPDC1B* and *WHSC1L1*, both regulating the cell survival and transformation of two different cell lines harboring the 8p amplicon. These results were also confirmed by long-term knockdown expression analysis with short hairpin RNA (shRNA). *PPAPDC1B* encodes a transmembrane protein phosphatase, a potential therapeutic target. We therefore explored its potential role in tumor growth *in vivo* using a siRNA approach in a xenograft model. We also investigated the mechanisms and pathways deregulated by PPAPDC1B in tumor progression by carrying out microarray experiments following *PPAPDC1B* knockdown with shRNAs.

Materials and Methods

Breast tumor samples and cell lines. We used frozen samples of 152 primary infiltrating ductal breast carcinomas (1 T0, 80 T1, 63 T2, 7 T3, and 1 T4b) and 11 normal breast tissues. Normal breast samples were obtained during reduction mammoplasty. Samples of tumor or normal breast tissues were flash frozen and stored at −80°C. All tumor samples contained >50% cancer cells and the normal samples contained >50% breast epithelial cells. This study was approved by the Institutional Review Boards of Institut Curie. In total, 19 breast cancer–derived cell lines (184B5, BT474, BT549, CAMA-1, HCC1937, Hs578T, MCF7, MDA-MB-134VI, MDA-MB-175VII, MDA-MB-231, MDA-MB-361, MDA-MB-415, MDA-MB-435S, MDA-MB-436, MDA-MB-453, MDA-MB-468, SKBR3, T47D, ZR-75-1, and ZR-75-30), one chemically transformed breast cell line (184B5), and one normal breast cell line (MCF 10A) were obtained from the American Type Culture Collection or from the German Resource Centre for Biological Material. The mouse fibroblastic cell line NIH-3T3 was kindly provided by Dr. S. Bellusci (Saban Research Institute, Los Angeles, CA).

DNA and RNA extraction from breast tumor samples and cell lines. DNA and RNA were extracted from frozen samples of breast carcinomas and normal breast by the cesium chloride protocol (20, 21). RNA was isolated from cell lines with Trizol (Sigma) for Affymetrix DNA chip experiments or RNeasy Mini kits (Qiagen) after siRNA/shRNA treatment for quantitative PCR experiments, whereas DNA was extracted with a conventional phenol-chloroform procedure.

CGH array. The 3.4K CIT (Carte d'Identité des Tumeurs program of the Ligue Nationale Contre le Cancer) bacterial artificial chromosome array and the experimental procedures for spotting, hybridization, and washing were as previously described (22).

DNA microarrays and probe set filter criteria. Microarray experiments were performed as previously described (23). For tumors, we used the Affymetrix Human Genome U133 set (HG-U133 A and B), consisting of two GeneChip arrays and containing 44,692 probe sets, corresponding to >16,000 unique genes for DNA microarray analysis. For cell lines, we used the Affymetrix Human Genome U133 Plus 2.0 DNA microarray containing 54,319 probe sets corresponding to ~19,000 unique genes. The CGH and expression data sets used in this study are available online.

Cell culture. NIH-3T3, CAMA-1, and MCF7 cells were cultured in DMEM supplemented with 10% FCS and 2 mmol/L L-glutamine; ZR-75-1 cells were grown in RPMI 1640 supplemented with 10% FCS, 1 mmol/L sodium pyruvate, 4.5 g/L glucose, and 10 mmol/L HEPES. Media were supplemented with 100 units/mL penicillin and 100 μg/mL streptomycin. All reagents were purchased from Life Technologies.

RNA interference. Transient transfections were performed using Oligofectamine according to the manufacturer's instructions (Invitrogen), with 100 nmol/L siRNA. A negative control siRNA (UUCUCCGAACGUGUCACGUdTdT) and siRNAs specific for *LSM1* (GCAUUGAUCAAUUUGCAAAdTdT), *BAG4* (ACAUAUACUUCAUGUGUAAdTdT), *DDHD2* (CAGUAUUGCUCGUCCUCAAdTdT), *PPAPDC1B* (*PPAPDC1B* siRNA: GCCUUGCCCUGGCUCUGAAdTdT; *PPAPDC1B* siRNA2: GAUGUUUGUUAUUGCAUUUdTdT), and *WHSC1L1* (GGGUAUCCAUCAUCAAUCAdTdT) were purchased from Qiagen. For each gene, the siRNA was designed to knock down all known mRNA isoforms.

Lentivirus-mediated shRNA knockdown of gene expression. We knocked down the expression of human *WHSC1L1* and *PPAPDC1B* genes in ZR-75-1 and CAMA-1 breast cancer cell lines using the Mission TRC human shRNA clone sets (pLKO.1-puro, Sigma). The sequences of the shRNAs used were CCGGCCATCATCAATCAGTGTGTATCTCGAGATACACACTGATTGATGATGGTTTTT (shRNA738, targeting exon 2) and CCGGGCTTCCATTACGATGCACAAACTCGAGTTTGTGCATCGTAATGGAAGCTTTTT (shRNA1959, targeting exon 6) for *WHSC1L1* and CCGGCCTTTCTGTCACCTCTACTTTCTCGAGAAAGTAGAGGTGACAGAAAGGTTTTTG (shRNA558, targeting exon 6) and CCGGGACACAAGAGACAGCAGACAACTCGAGTTGTCTGCTGTCTCTTGTGTCTTTTTG (shRNA222, targeting exon 3) for *PPAPDC1B*. For each gene, the shRNAs were designed to knock down all known mRNA isoforms. Lentivirus was produced by transfecting 293T cells with the pLKO.1-puro construction by the calcium phosphate method. For cell infection, viral supernatants were supplemented with 8 μg/mL polybrene and incubated with cells for 16 h. Cells expressing shRNA were selected on puromycin (2 μg/mL) for 3 wk for functional studies (cell proliferation and colony formation assays) and for 4 to 10 d after infection for RNA extraction and Affymetrix DNA array studies.

Quantitative real-time reverse transcription-PCR. Reverse transcription was performed with 1 μg of total RNA, random hexamer primers (20 pmol), and 200 units of avian myeloblastosis virus reverse transcriptase. Assays-on-Demand for assessing expression levels for the *LSM1, WHSC1L1, BAG4, DDHD2*, and *PPAPDC1B* genes and for the control *TATA-binding protein* (*TBP*) gene were obtained from Applied Biosystems. PCR was carried out in an ABI PRISM 7900 real-time thermal cycler using the Taqman master mix (Applied Biosystems).

Proliferation assay. Cells were plated (3×10^4 per well) in a 24-well plate and transfected with 100 nmol/L siRNA. They were treated with trypsin 72 h after transfection and stained with trypan blue, and viable cells were counted in triplicate using a Malassez hematocytometer.

Terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling assay. Cells (3×10^4 per well) were seeded on a glass slide in a 24-well plate and transfected with 100 nmol/L siRNA. DNA fragmentation was evaluated 72 h after transfection using a terminal deoxynucleotidyl transferase–mediated dUTP nick end labeling (TUNEL) assay detection kit (Roche Diagnostics) according to the manufacturer's instructions. We analyzed 600 cells under a light microscope, determining the proportion of labeled cells.

Focus formation assay. For MCF7, CAMA-1, and ZR-75-1, 5×10^4 cells per well were plated in a six-well plate and transfected with 100 nmol/L siRNA. Cells cultured for 15 d in medium changed every 3 d were washed twice with ice-cold PBS, fixed by incubation for 10 min with ice-cold methanol, and stained with 0.5% crystal violet for colony scoring.

Soft agar assay. For MCF7, CAMA-1, and ZR-75-1, 20,000 siRNA-transfected cells in DMEM or RPMI 1640 supplemented with 10% FCS and 0.3% agar were added to triplicate wells containing medium and 0.8% agar on 12-well plates. The plates were incubated for 12 d and colonies with diameters of >50 μm were scored as positive using a phase-contrast microscope equipped with a measuring grid. For NIH-3T3 cells, we added 20,000 transfected cells, in triplicate, to each well of a 12-well plate. Each well contained DMEM supplemented with 10% newborn calf serum and solidified with 0.3% agar. Colonies were counted after 2 wk, as described above.

Formation of siRNA/atelocollagen complex. Atelocollagen is a highly purified type I collagen obtained from calf dermis by pepsin treatment (Koken Co. Ltd.). We prepared siRNA/atelocollagen complexes as previously described (24) by mixing an equal volume of atelocollagen (in PBS at pH 7.4) and siRNA solution and incubating overnight at 4°C.

Tumor formation in nude mice. Ten 6-wk-old female Swiss *nu/nu* mice were obtained from Charles River Laboratories and reared in the animal

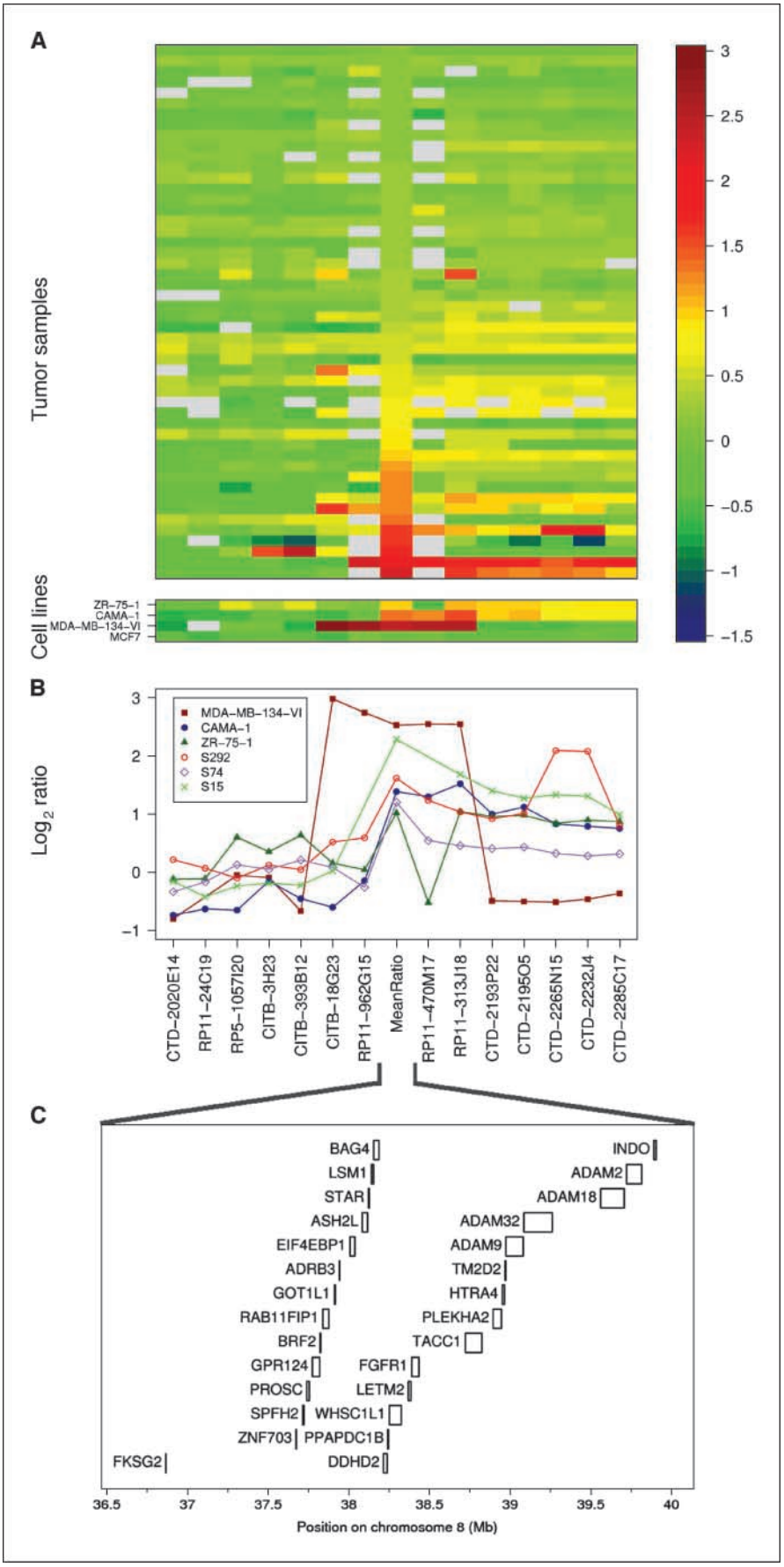


Figure 1. Genetic aberrations on chromosome 8p11-12 in breast cancers. *A,* CGH array of the 8p11-12 chromosome in breast cancer samples (*top*) and cell lines (*bottom*). Each column represents a clone on the array. Clones are ordered according to genome position based on the NCBI human genome reference sequence 36 (May 2006) from telomere (*left*) to centromere (*right*). Three clones were localized to the same position (CTD-2014P5, CTD-2139K9, and CTD-2092C4). We therefore considered, for this position, the mean signal for the three clones: mean ratio. Log_2 values for the Cy5 (tumor DNA)/Cy3 (control DNA) ratio are depicted on a color scale in which blue indicates loss, red indicates gain, and gray indicates an absence of data. *B,* genomic profiles of 8p11-12 amplification in selected cell lines or tumor samples, defining a common region of amplification between clone RP11-962G15 (chr8:36653064-36836067) and clone RP11-470M17 (chr8:40010617-40185770). *C,* known genes present in the common region of amplification based on build 36 from NCBI.

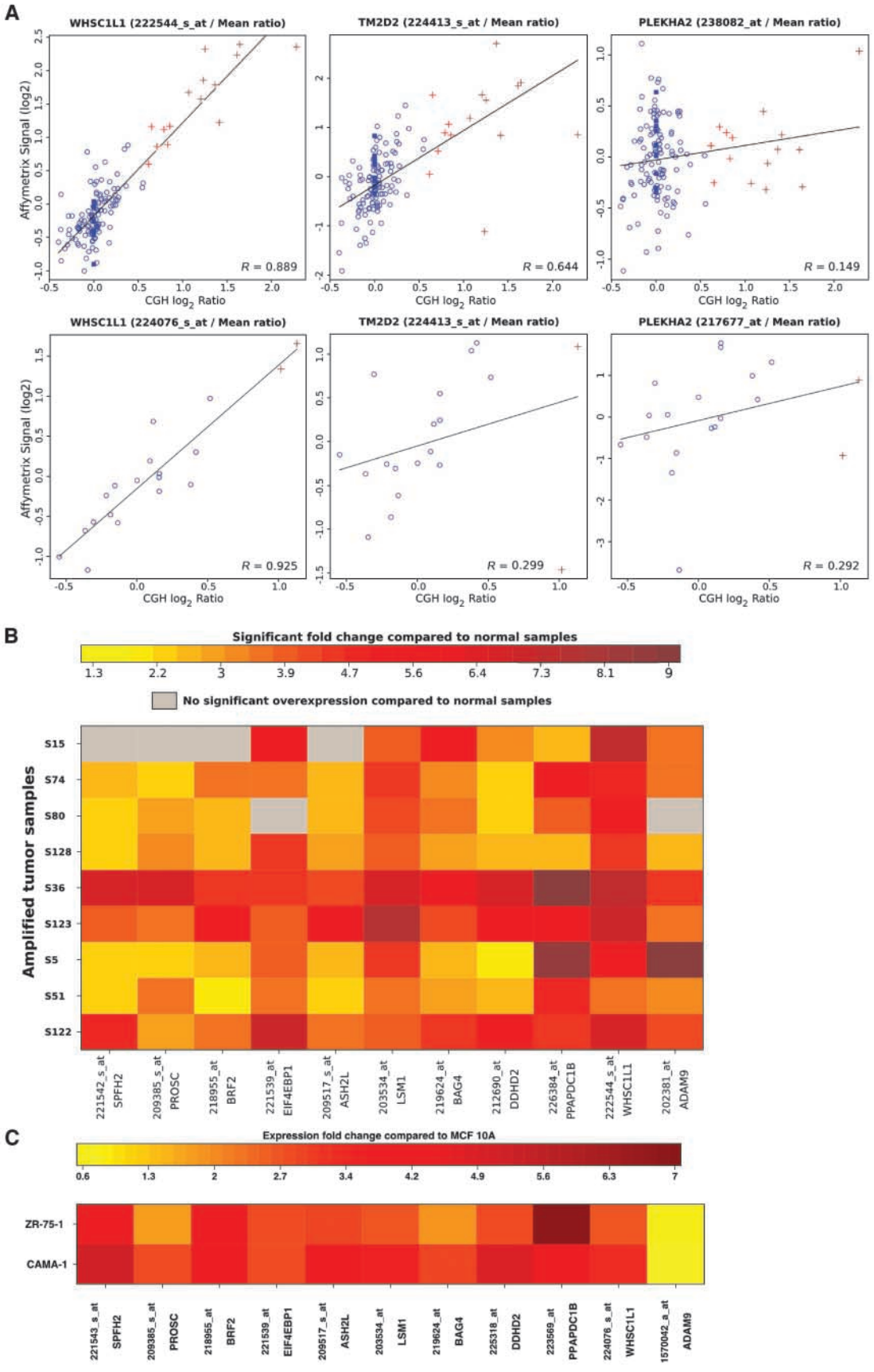


Table 1. Pearson's correlation between DNA copy number and gene expression for the 28 genes of the minimal 8p region of amplification

	Cell lines		Tumors	
	Affymetrix U133 Plus 2 probe set	*R*	Affymetrix U133 probe set	*R*
FKSG2	208588_at	0.372	208588_at	0.126
ZNF703	222760_at	0.210	222760_at	0.458
SPFH2	221543_s_at	0.771	221542_s_at	0.675
PROSC	209385_s_at	0.929	209385_s_at	0.714
GPR124	65718_at	0.305	65718_at	−0.028
BRF2	218955_at	0.641	218955_at	0.650
RAB11FIP1	219681_s_at	0.347	225177_at	0.678
GOT1L1	1553879_a_at	0.305	NA	
ADRB3	206812_at	0.146	206812_at	0.057
EIF4EBP1	221539_at	0.677	221539_at	0.578
ASH2L	209517_s_at	0.921	209517_s_at	0.706
STAR	204548_at	−0.310	204548_at	−0.060
LSM1	203534_at	0.864	203534_at	0.671
BAG4	222909_s_at	0.803	228189_at	0.709
DDHD2	225318_at	0.918	212690_at	0.808
PPAPDC1B	223569_at	0.552	226384_at	0.744
WHSC1L1	224076_s_at	0.925	222544_s_at	0.889
LETM2	1552546_a_at	0.209	NA	
FGFR1	222164_at	0.380	210973_s_at	0.603
TACC1	1554690_a_at	0.497	242290_at	0.139
PLEKHA2	217677_at	0.292	238082_at	0.149
HTRA4	1553706_at	−0.228	NA	
TM2D2	224413_s_at	0.299	224413_s_at	0.644
ADAM9	1570042_a_at	0.433	202381_at	0.530
ADAM32	1552266_at	0.235	217090_at	0.062
ADAM18	1568970_at	0.013	207597_at	0.088
ADAM2	207664_at	0.086	207664_at	0.015
INDO	1568638_a_at	0.201	210029_at	−0.115

NOTE: *R* values of >0.4 ($P < 0.05$) are highlighted in gray. If $R > 0.4$ in both cell lines and tumors, the gene name is highlighted in gray. If several probe sets were available for one gene, we used only the probe set yielding the highest correlation coefficient to ensure that no candidate gene was spuriously excluded.
Abbreviation: NA, probe set not available.

facility of the Curie Institute in specified pathogen-free conditions. Animals were housed and cared for in accordance with the institutional guidelines of the French National Ethics Committee (Ministère de l'Agriculture et de la Pêche, Direction Départementale des Services Vétérinaires, Paris, France) under the supervision of investigators with the required accreditation. Slow-release estradiol pellets (1.7 mg, 90-d release; Innovative Research of America) were implanted s.c. in these mice 3 d before tumor transplantation. We injected 5×10^6 ZR-75-1 cells per site s.c. into each flank (dorsal region) of each mouse. When tumors reached about 20 mm^3 (after 15 d), mice were randomly assigned to one of two groups of five mice each and were treated with control or *PPAPDC1B*-specific siRNAs complexed with 0.5% atelocollagen. The siRNA/atelocollagen complex was injected directly into the tumors once per week (4 μg of control siRNA or 2 μg *PPAPDC1B* siRNA + 2 μg *PPAPDC1B* siRNA2/50 μL/tumor).

Tumor formation was monitored for up to 48 d and tumor size was determined twice weekly with Vernier calipers: two perpendicular diameters were used to estimate tumor volume according to the formula $ab^2/2$, where a is the largest diameter and b is the smallest diameter.

Figure 2. Identification of candidate driver genes within the minimal region of amplification on 8p11-12 based on expression data. *A,* linear regression analysis of the 128 breast carcinomas (*top*) and of the 19 cell lines (*bottom*) for which we had both CGH and expression data. The Affymetrix signal and the CGH $\log_2$ ratio were compared using Pearson's correlation test. Expression levels were considered significantly modified with respect to DNA copy number if $R > 0.4$ ($P < 0.05$; Table 1). Genes were considered to be candidate driver genes if $R \geq 0.4$ in both cell lines and tumors (as *WHSC1L1*). Genes were excluded if $R < 0.4$ in cell lines (such as *TM2D2*), tumors, or both (such as *PLEKHA2*). *B* and *C,* comparison of gene expression levels between samples presenting DNA amplification at 8p11-12 and normal samples (*B*) and between cell lines presenting 8p amplification and MCF 10A, a normal breast epithelium-derived cell line (*C*). Each row corresponds to one amplified tumor sample/cell line and each column to one gene ordered according to its genomic location, from telomere (*left*) to centromere (*right*). *B,* for genes with significantly higher levels of expression in a given sample than in normal samples (z score > 2; $P < 0.0214$; see Materials and Methods), the fold difference with respect to mean expression in normal samples is depicted on a color scale (*yellow* to *red*). Genes are otherwise shown in light gray. Case S15 is particularly informative because it excludes four genes (*SPFH2, PROSC, BRF2*, and *ASH2L*) as potential candidate driver genes because these genes are not overexpressed as a result of the observed 8p11-12 amplification. Similarly, case S80 excludes *EIF4EBP1* and *ADAM9* as possible driver genes. In cases in which several probe sets were available for a gene, we selected the set displaying differential expression in the largest number of samples to ensure that no candidate gene was spuriously excluded.

Production of *PPAPDC1B*-expressing NIH-3T3 clones. The *PPAPDC1B* cDNA (cDNA clone IMAGE:5267610) in the pBluescriptR vector was purchased from Geneservice and the sequence was verified by sequencing. This cDNA was excised from the vector and inserted into pcDNA3.1. NIH-3T3 cells were transfected with empty pcDNA3.1 or with *PPAPDC1B*-pcDNA3.1 using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. G418 (400 μg/mL) was added to culture medium 48 h after transfection, and drug selection was continued for 2 wk. Single colonies were ring cloned and expanded. Two control clones were obtained (Neo1 and Neo2) and three *PPAPDC1B*-expressing clones presenting different levels of *PPAPDC1B* mRNA were selected.

Statistics. For each gene in the common amplification region, the RNA levels measured by Affymetrix arrays of the 9 8p-amplified samples were compared with those of 114 tumor samples with an unchanged 8p-DNA copy number and with those of normal samples using the Wilcoxon rank sum test. The Affymetrix signal and CGH $\log_2$ ratio were compared using Pearson's correlation test. For each gene with an R value of >0.4 ($P < 0.05$), the level of RNA in each amplified tumor sample was compared with the distribution of RNA levels for the same gene in normal samples. The difference was considered significant if it exceeded two SDs (z score > 2; $P < 0.0214$). We evaluated the results of the functional experiments using χ^2 tests, Student's t test, or the Wilcoxon rank sum test depending on the experiment considered. Differences were considered significant if $P < 0.05$. Affymetrix expression data for *PPAPDC1B* knockdown experiments in cell lines were analyzed using Student's t test and the same significance threshold as above. The genes identified as differentially expressed by this method for each shRNA were listed in order of P values and $\log_2$ fold change sign. The lists were then compared using hypergeometric tests and Bayesian methodology (25) to evaluate their concordance for the two shRNAs.

Results

We used CGH on DNA microarrays (array CGH) to map regions of amplification (identified based on a normalized array CGH $\log_2$ ratio higher than 1.0) in 152 ductal breast carcinomas and 21 cell lines. Consistent with previous studies, the most frequently amplified regions were 17q13 (13%), 8p11-12 (9%), and 11q13 (10%), and the most frequently lost regions were 16q (50%), 8p (40%), and 11q (38%). We focused our study on the chromosome 8 amplicon and delineated a 3.2-Mb region of DNA amplification on chromosome 8p11-12 common to 11 of the 152 infiltrating ductal breast carcinomas (7.2%) and to 3 of the 21 breast cancer cell lines (14%) studied (Fig. 1*A* and *B*). This region encompassed the DNA fragments from clone RP11-962G15 to clone RP11-470M17 (Fig. 1*A* and *B*) and contained 28 known genes according to the Build 36 Human Reference Sequence from National Center for Biotechnology Information (NCBI; May 2006; Fig. 1*C*).

Affymetrix DNA microarrays were used to analyze RNA levels in 128 of the 152 breast carcinomas, in 18 of the 21 cell lines studied on CGH arrays, and in 11 normal breast samples. Affymetrix data were thus obtained for 9 of the 11 tumors and two of the three cell

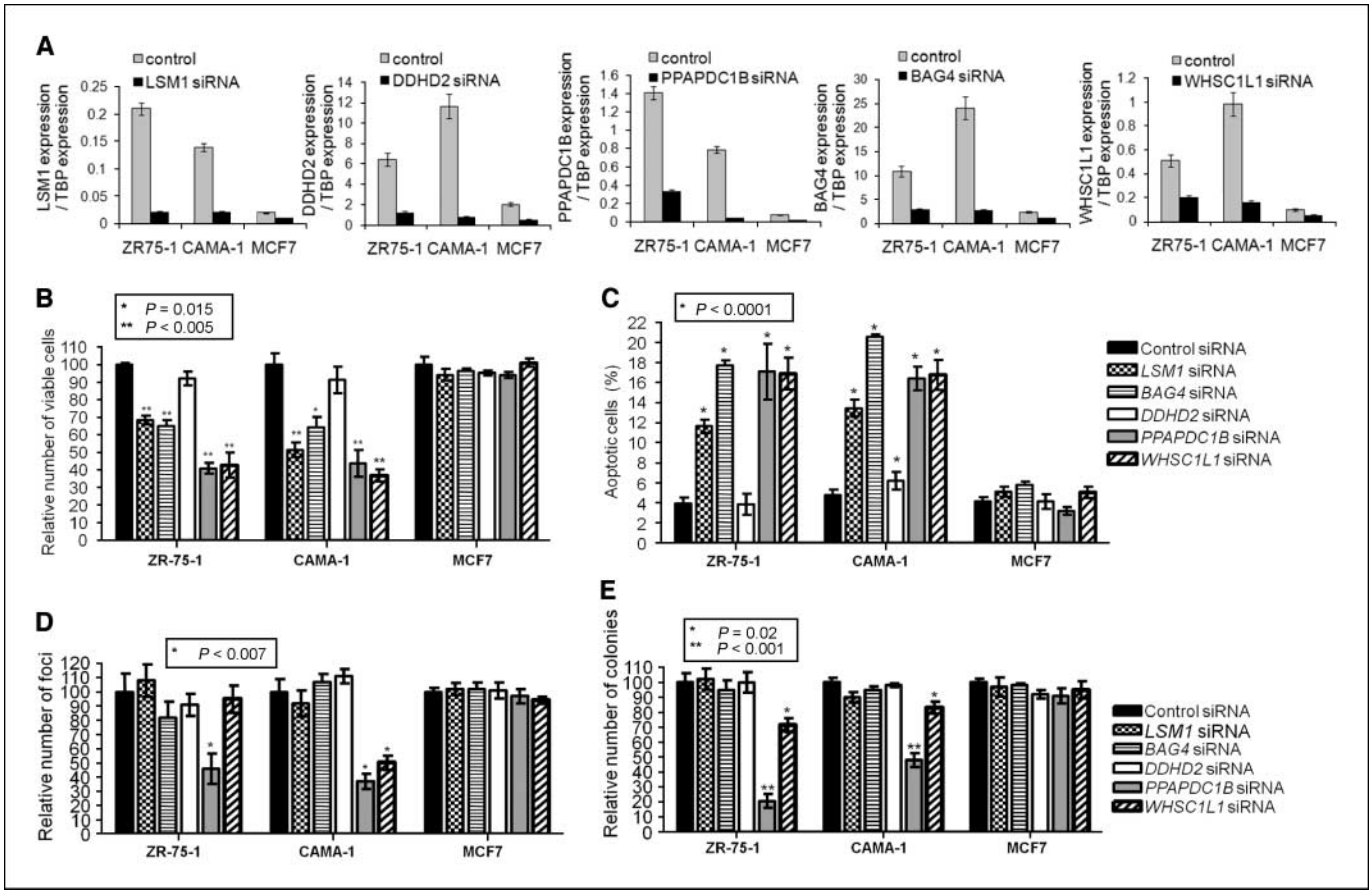


Figure 3. *PPAPDC1B* and *WHSC1L1* regulate both the survival and transformation of 8p11-12–amplified breast cancer cells. *A,* results are from a representative experiment. *B* and *C,* effect of candidate driver gene knockdown on cell viability (*B*) and cell apoptosis (*C*), as assessed by TUNEL assay. *D* and *E,* effect of candidate driver gene knockdown on focus-forming ability (*D*) and anchorage-independent colony formation (*E*). *Columns,* mean of two independent experiments carried out in triplicate; *bars,* SD.

lines for which CGH array-based evidence of 8p11-12 amplification was obtained. Eleven of the 28 genes encompassed by the common region of amplification presented a direct relationship between DNA copy number and expression level in both tumors and cell lines, with a correlation coefficient $R > 0.4$ ($P < 0.05$), indicating that DNA copy number had a significant effect on gene expression level (Fig. 2*A*; Table 1; Supplementary Fig. S1). Accordingly, these 11 genes were more strongly expressed in tumors with 8p amplification than in tumors without amplification or in normal breast tissue, and in breast cancer cell lines with 8p amplification than in nonamplified cell lines (Supplementary Table S1). Consistent results were obtained for all Affymetrix probe sets, when available, for a given gene (data not shown). Given that candidate driver genes should be systematically overexpressed owing to gene amplification, we were able to exclude 6 of these 11 genes based on data from two particularly informative cases (S15 and S80; Fig. 2*B*). Indeed, the *SPFH2, PROSC, BRF2*, and *ASH2L* genes were not always significantly overexpressed (Fig. 2*B*) despite the high level of amplification of the 8p11-12 region observed in S15 (Fig. 1*B*). Similarly, the *EIF4EBP1* and *ADAM9* genes could be excluded based on the data for sample S80 (Fig. 2*B*). *ADAM9* was also excluded based on the data for ZR-75-1 and CAMA-1, as it was not more strongly expressed in these cell lines with 8p amplification than in MCF10A, a normal breast epithelium–derived cell line (Fig. 2*C*). The 14 other genes of the region, which presented no significant correlation between DNA and RNA levels in cell lines and tumors and for which we had Affymetrix data for the tumors, were not systematically overexpressed in amplified tumors (Supplementary Fig. S2). Five genes, *LSM1, BAG4, DDHD2, PPAPDC1B*, and *WHSC1L1*, were therefore identified as candidate drivers by this genomic and transcriptomic analysis of breast tumors.

RNA interference technology (26) was then used to investigate the role of these five genes in the cell growth and tumorigenic properties of two cell lines with 8p11-12 amplification (CAMA-1 and ZR-75-1) and in one control cell line without this amplicon (MCF7). The transfection of CAMA-1 and ZR-75-1 cells with specific siRNAs markedly decreased mRNA levels, resulting in levels similar to those observed in untransfected MCF7 cells (70–90% inhibition; Fig. 3*A*). The silencing of each gene individually had no significant effect on the levels of expression of the other four genes (data not shown). The specific knockdown of *LSM1, BAG4, PPAPDC1B*, or *WHSC1L1* yielded fewer viable ZR-75-1 and CAMA-1 cells than transfection with the control siRNA (Fig. 3*B*). None of these siRNAs had any effect on MCF7 cells, suggesting that the effect observed following transfection with a specific siRNA was due to the silencing of a specific gene rather than to an off-target effect (Fig. 3*B*). This decrease in the number of viable cells could be attributed to an increase in the rate of apoptosis, with no significant change in cell cycle progression (Fig. 3*C*; data not shown). Thus, each of these four genes seems to regulate cell survival in breast carcinomas with 8p amplification. However, they differ in terms of their role in cloning efficiency. Indeed, *PPAPDC1B* silencing significantly decreased the colony-forming ability of CAMA-1 and ZR-75-1 cells in both anchorage-dependent (focus formation on plates) and anchorage-independent (in soft agar) conditions, whereas it had no effect on MCF7 cells (Fig. 3*D* and *E*). *WHSC1L1* knockdown also inhibited focus formation by CAMA-1 cells (Fig. 3*D*) and, to a lesser extent, the growth of both CAMA-1 and ZR-75-1 cells in soft agar (Fig. 3*E*). In contrast, the silencing of *LSM1, BAG4*, and *DDHD2* had no effect on the cloning efficiency of cells with and without 8p11-12 amplification.

Our results, obtained with a siRNA approach, suggest that *PPAPDC1B* and *WHSC1L1* are two major driver genes of the 8p11-12 amplicon. Both are strongly overexpressed in breast carcinomas with 8p amplifications and regulate cell survival and transformation. The ability of these two major genes to regulate 8p-amplified breast cancer cell proliferation/survival and cell transformation was confirmed by long-term knockdown of the expression of these genes following the stable expression of shRNAs in ZR-75-1 and CAMA-1 cell lines (Supplementary Fig. S3), fully confirming the effects observed with siRNAs. Indeed, both *WHSC1L1* and *PPAPDC1B* shRNAs efficiently knocked down *WHSC1L1* and *PPAPDC1B* transcript levels by 80% to 90% (Supplementary Fig. S3*A*). Cells expressing *WHSC1L1* or *PPAPDC1B* shRNAs grew much slower than control cells, resulting in a 4-fold difference in the number of shRNA-expressing cells 2 weeks after seeding (Supplementary Fig. S3*B*). Moreover, cells expressing *WHSC1L1* or *PPAPDC1B* shRNAs formed fewer colonies in focus formation assay than control cells (Supplementary Fig. S3*C*).

However, as *PPAPDC1B* knockdown had a greater effect on cloning efficiency than *WHSC1L1* knockdown, and as the phosphatase domain of PPAPDC1B may be an appropriate

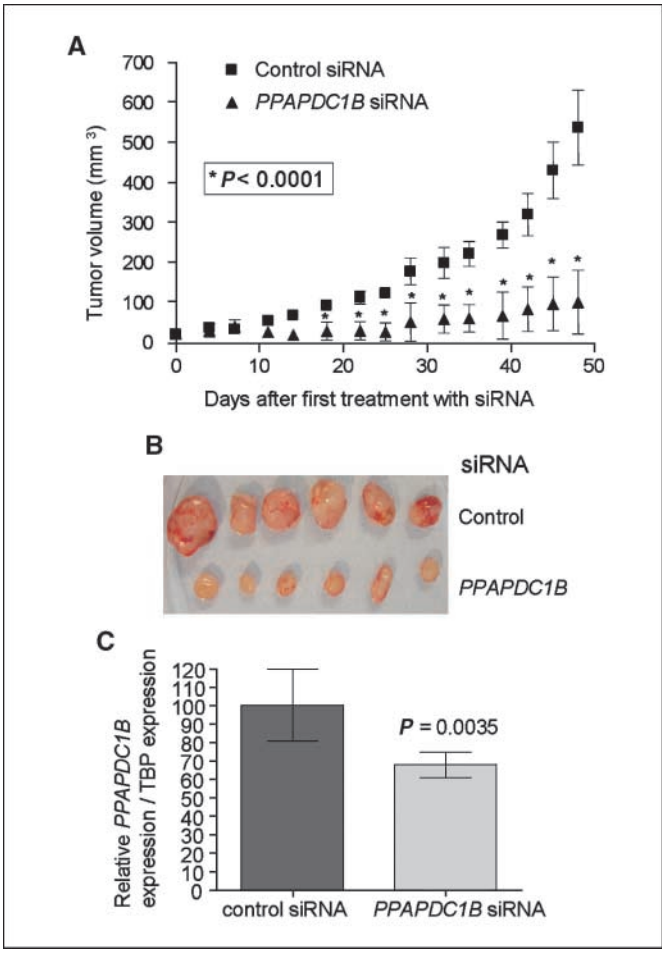
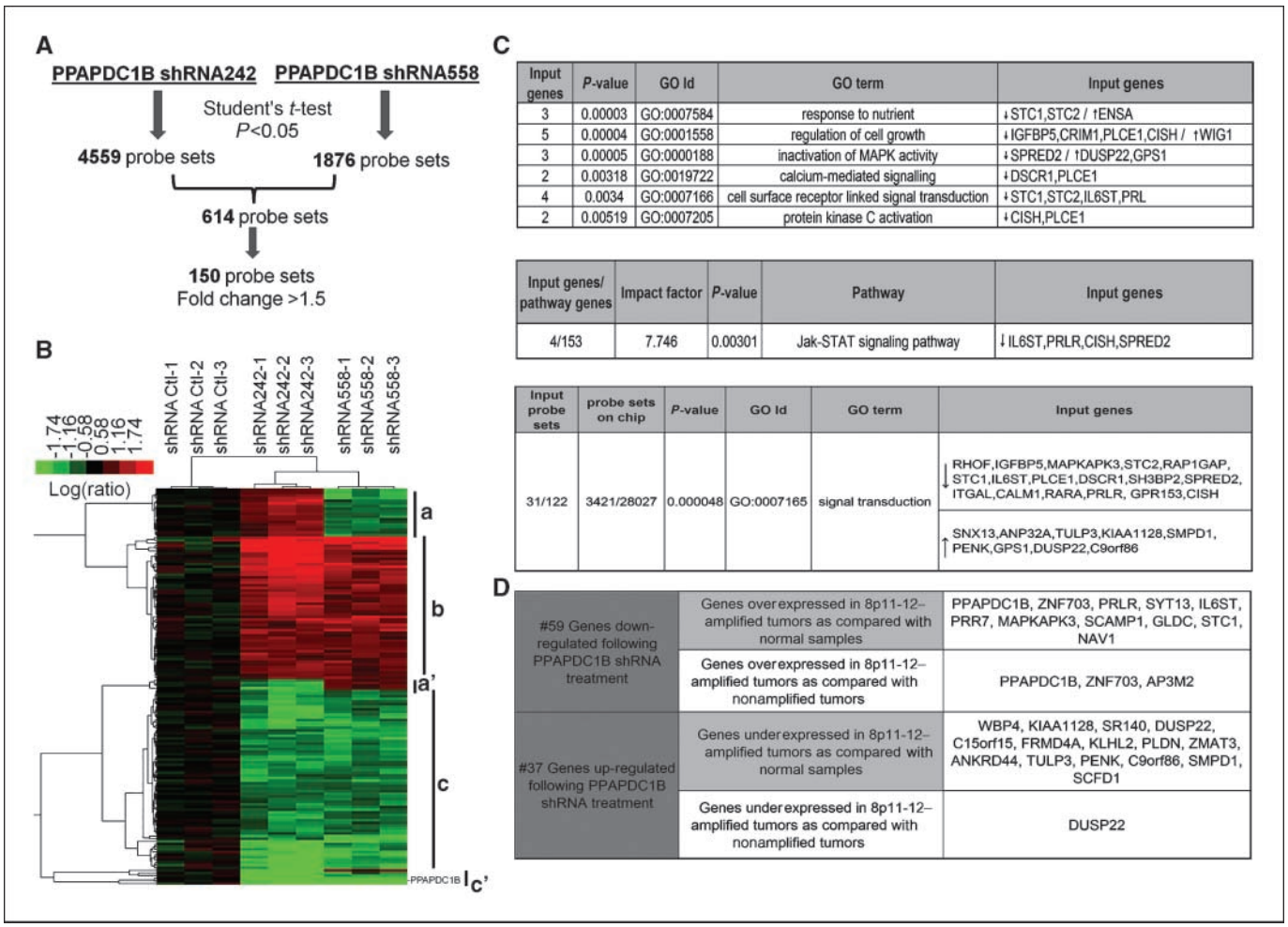


Figure 4. *PPAPDC1B* regulates the tumorigenicity of breast cancer cells *in vivo*. Effect of the *PPAPDC1B* siRNA/atelocollagen complexes on the growth of xenografted ZR-75-1 tumors. *A,* tumor-bearing mice were treated weekly with control siRNA or a mixture of two *PPAPDC1B*-specific siRNAs complexed with atelocollagen (5 mice and 10 tumors per group; the first injection corresponds to day 0). *B,* tumors observed after 48 d. *C, PPAPDC1B* mRNA levels divided by *TBP* mRNA levels ± SD in treated and control tumors, as assessed by quantitative PCR.



C

Input genes	P-value	GO Id	GO term	Input genes
3	0.00003	GO:0007584	response to nutrient	↓STC1,STC2 / ↑ENSA
5	0.00004	GO:0001558	regulation of cell growth	↓IGFBP5,CRIM1,PLCE1,CISH / ↑WIG1
3	0.00005	GO:0000188	inactivation of MAPK activity	↓SPRED2 / ↑DUSP22,GPS1
2	0.00318	GO:0019722	calcium-mediated signalling	↓DSCR1,PLCE1
4	0.0034	GO:0007166	cell surface receptor linked signal transduction	↓STC1,STC2,IL6ST,PRL
2	0.00519	GO:0007205	protein kinase C activation	↓CISH,PLCE1

Input genes/ pathway genes	Impact factor	P-value	Pathway	Input genes
4/153	7.746	0.00301	Jak-STAT signaling pathway	↓IL6ST,PRLR,CISH,SPRED2

Input probe sets	probe sets on chip	P-value	GO Id	GO term	Input genes
31/122	3421/28027	0.000048	GO:0007165	signal transduction	↓RHOF,IGFBP5,MAPKAPK3,STC2,RAP1GAP, STC1,IL6ST,PLCE1,DSCR1,SH3BP2,SPRED2, ITGAL,CALM1,RARA,PRLR, GPR153,CISH
					↑SNX13,ANP32A,TULP3,KIAA1128,SMPD1, PENK,GPS1,DUSP22,C9orf86

D

#59 Genes down-regulated following PPAPDC1B shRNA treatment	Genes overexpressed in 8p11-12–amplified tumors as compared with normal samples	PPAPDC1B, ZNF703, PRLR, SYT13, IL6ST, PRR7, MAPKAPK3, SCAMP1, GLDC, STC1, NAV1
	Genes overexpressed in 8p11-12–amplified tumors as compared with nonamplified tumors	PPAPDC1B, ZNF703, AP3M2
#37 Genes up-regulated following PPAPDC1B shRNA treatment	Genes underexpressed in 8p11-12–amplified tumors as compared with normal samples	WBP4, KIAA1128, SR140, DUSP22, C15orf15, FRMD4A, KLHL2, PLDN, ZMAT3, ANKRD44, TULP3, PENK, C9orf86, SMPD1, SCFD1
	Genes underexpressed in 8p11-12–amplified tumors as compared with nonamplified tumors	DUSP22

Figure 5. PPAPDC1B interferes with signal transduction pathways. Changes in transcript levels 96 h after infection of the 8p11-12–amplified ZR-75-1 cell line with lentiviruses encoding shRNA, as assessed using Affymetrix U133 Plus 2.0 DNA microarrays. Three experiments were carried out for each shRNA tested (shRNA Ctl, *PPAPDC1B* shRNA242, and *PPAPDC1B* shRNA558). We considered 28,027 probe sets with a Present MAS5 call on at least two of the nine chips for the study. *A,* identification of genes specifically altered by *PPAPDC1B* knockdown. For each *PPAPDC1B* shRNA, the significance of differences in expression with respect to the control shRNA was assessed by carrying out Student's *t* tests. We considered 614 probe sets altered by both shRNAs to be modulated by *PPAPDC1B* knockdown ($P < 0.05$), the others probably being off-target transcripts. We found that 150 of these probe sets presented an average change in expression of >1.5-fold with both shRNAs. *B,* heat map visualization of these 150 regulated transcripts (*Y axis*) in three experiments for each shRNA used (*X axis*). The expression ratio was determined for each shRNA by comparison with the mean level of expression on shRNA Ctl chips. Two clusters of nonspecifically regulated genes (*a* and *a'*) were identified, together with one cluster of up-regulated genes (*b*) and two clusters of down-regulated genes (*c* and *c'*). *C,* signaling pathways implicated by *PPAPDC1B* knockdown. One hundred twenty-two probe sets/96 genes displaying significant and specific changes in expression following *PPAPDC1B* knockdown were analyzed using Onto-Express (*top*) and Pathway-Express (*middle*) software to identify, respectively, enrichment in GO biological process annotations and functional pathways altered as a result of *PPAPDC1B* knockdown. Results were sorted by *P* value ($P < 0.01$). *Bottom,* the GO annotation of biological process "signal transduction" is a branch with 325 leaves/GO annotations in the GO tree. Each probe set presenting one of these "leaves" was attributed to this process and enrichment in this process among genes modulated by *PPAPDC1B* was evaluated with Fisher's exact test. *D,* for each *PPAPDC1B*-modulated gene (59 genes down-regulated and 37 up-regulated following PPAPDC1B shRNA treatment), the significance of differences in expression between 8p11-12–amplified tumors and other breast tumors or normal samples was assessed using Wilcoxon rank sum tests. Genes displaying a significant modification of expression in 8p11-12 tumors ($P < 0.05$) are indicated.

pharmacologic target, we decided to focus our study on the role of this gene in breast cancer cell aggressiveness *in vivo*. ZR-75-1 cells were implanted s.c. in athymic nude mice. Once tumors were established, mice were randomly selected for treatment with control or *PPAPDC1B*-specific siRNAs, both complexed with atelocollagen, a reagent shown to increase mRNA knockdown efficiency *in vivo* (24). We used a combination of two different siRNAs targeting two different exons of *PPAPDC1B* (exons 2 and 3), as this approach is more efficient for gene silencing than the use of a single siRNA (27). The second anti-*PPAPDC1B* siRNA (siRNA2) knocked down mRNA levels and decreased the ability of CAMA-1 and ZR-75-1 cells to form colonies in anchorage-independent conditions as efficiently as the first (data not shown). After 48 days of treatment, tumor volume was 80% lower in mice treated with *PPAPDC1B* siRNAs than in mice treated with control siRNAs ($P < 0.001$, Wilcoxon rank sum test; Fig. 4*A* and *B*). No histologic difference was observed between treated and control tumors (data not shown). This inhibition of tumor growth was associated with a significant decrease in *PPAPDC1B* mRNA levels ($P = 0.0035$, Student's *t* test; Fig. 4*C*).

Our results clearly identify *PPAPDC1B* as a major gene within the 8p11-12 amplicon in breast carcinoma regulating cell survival and cell transformation both *in vitro* and *in vivo*, suggesting that PPAPDC1B may play an oncogenic role. The potentially oncogenic properties of this protein were further shown by the induction of NIH-3T3 cell transformation by stable *PPAPDC1B* expression (Supplementary Fig. S4*A* and *B*). Indeed, three independent *PPAPDC1B*-expressing clones formed colonies in anchorage-independent conditions,

whereas control clones formed far fewer, smaller colonies (Supplementary Fig. S4*B*).

Our results, based on both the knockdown and expression of *PPAPDC1B* in different cell lines, clearly show that the product of this gene is involved in cell survival and transformation. We investigated the mechanism by which *PPAPDC1B* regulates these processes in a 8p11-12 amplified cell line (ZR-75-1) by carrying out expression profiling experiments with Affymetrix U133 Plus 2.0 DNA microarrays following *PPAPDC1B* knockdown with two different shRNAs (sh242 and sh558, targeting exon 3 and 6, respectively; Fig. 5*A*). The two ordered lists of genes displaying a significant change in expression following *PPAPDC1B* knockdown ($P < 0.05$, t test) overlapped considerably and significantly, as shown by hypergeometric test ($P < 10e{-}22$) and Bayesian methodology (data not shown; ref. 25). We found that 150 of the 614 probe sets displaying significant modulation by both *PPAPDC1B* shRNAs presented a more than 1.5-fold change in expression. These 150 probe sets formed five clusters: two small nonspecific clusters containing genes in which expression levels changed in opposite directions with the two shRNAs (28 probe sets/19 genes), one cluster of up-regulated genes (48 probe sets/37 genes), and two clusters of down-regulated genes (74 probe sets/59 genes; Fig. 5*B*). The last three of these clusters, corresponding to 122 probe sets displaying alterations (96 genes; Supplementary Table S2), were thus considered to be significantly and specifically altered (by both shRNAs, in the same direction in each case) by *PPAPDC1B* knockdown. They were analyzed further with Onto-Express (Fig. 5*C, top*) and Pathway-Express (Fig. 5*C, middle*) software (28–35) to identify enrichment in Gene Ontology (GO) biological processes and in functional pathways, respectively. This approach suggested the probable involvement of calcium-mediated signaling, mitogen-activated protein kinase (MAPK), protein kinase C (PKC), and Janus-activated kinase (JAK)-signal transducer and activator of transcription (STAT) pathways in PPAPDC1B-mediated tumorigenesis. Based on GO annotations, a more general significant enrichment ($P < 0.01$) in probe sets involved in signal transduction (Fig. 5*C, bottom*) was observed. We also investigated the genes that were both altered following *PPAPDC1B* knockdown and differentially expressed in 8p11-12–amplified tumors and either normal tissue (26 genes) or non–8p11-amplified samples (4 genes; Fig. 5*D*). Like *PPAPDC1B*, the other two genes down-regulated by *PPAPDC1B* knockdown and overexpressed in 8p11-12–amplified tumors belonged to the 8p11-12 amplicon, suggesting that *PPAPDC1B* regulation acts together with gene amplification to increase the expression levels of *ZNF703* and *AP3M2*. A finding of even greater importance was the demonstration that *DUSP22*, which encodes a dual-specificity phosphatase involved in various pathways, is both up-regulated by *PPAPDC1B* knockdown and underexpressed in 8p-amplified tumors (Fig. 5*D*).

Discussion

Oncogene activation through increased gene copy number contributes to malignant transformation in human cancers. The 8p11-12 chromosome region is one of the most frequently amplified genomic fragments in breast carcinoma. It therefore probably contains one or possibly several oncogenes.

By combining analyses of RNA levels and copy number changes for 152 ductal breast cancer samples and 21 breast cancer cell lines, we were able to identify five candidate oncogenes in the 8p11-12 region: *LSM1, BAG4, DDHD2, PPAPDC1B*, and *WHSC1L1*. These genes were identified based on significant correlations between expression levels and copy number and systematic and significant overexpression in all tumor samples and cell lines presenting 8p11-12 amplification with respect to their 8p11-12 normal counterparts. Several groups have reported analyses of gene expression as a function of copy number changes in the 8p11-12 region in breast carcinomas and have also identified candidate oncogenes based on overexpression as a consequence of amplification. Two of the genes we identified, *LSM1* and *PPAPDC1B*, were also identified as candidate oncogenes by several other groups (13–17, 36). *DDHD2* and *WHSC1L1* were identified by two groups (14, 15) and *BAG4* was identified by Garcia and colleagues (13), Yang and colleagues (15), and Still and colleagues (36). A systematic functional study by siRNA inactivation of the five candidate oncogenes identified in this study indicated that the silencing of *PPAPDC1B, WHSC1L1*, and, to a lesser extent, *LSM1* and *BAG4* impaired the clonogenicity and/or survival of breast cancer cell lines harboring 8p11-12 amplifications. The absence of a clear phenotype following the invalidation of these genes in the MCF7 cell line (with no 8p11-12 amplification) provides strong evidence for the gene-specific effects of these siRNAs and against significant off-target effects. Moreover, the targeting of *WHSC1L1* and *PPAPDC1B* by transfection with siRNAs and by infection with lentiviruses encoding two different shRNAs for each gene had very similar phenotypic consequences. The silencing of any one of the four genes identified promoted the apoptosis of 8p-amplified cell lines, but only *WHSC1L1* or *PPAPDC1B* silencing impaired colony formation. This suggests that the cell population is not homogeneous and that these last two genes regulate clonogenic cell survival, whereas *LSM1* and *BAG4* regulate only the survival of nonclonogenic cells.

WHSC1L1 has already been identified as an oncogene; it is amplified and overexpressed in lung carcinoma and its transforming abilities have been described in a lung carcinoma cell line (37). We confirm previous reports that *PPAPDC1B* is consistently overexpressed when amplified (13–17, 36) and provide the first functional evidence of its involvement in breast cancer cell transformation. Knockdown experiments with two different shRNAs in a *PPAPDC1B*-amplified cell line indicated that this gene interfered with critical signaling pathways, including calcium-mediated signaling, PKC, JAK-STAT, and MAPK pathways. The expression of *DUSP22*, which encodes a dual-specificity phosphatase that negatively regulates the estrogen receptor (ER) α signaling pathway (38), is both up-regulated by *PPAPDC1B* knockdown and down-regulated by 8p11-12 amplification, as shown by comparisons with other breast tumors and normal tissues. This suggests that *PPAPDC1B* amplification may increase ER activity in ER-positive tumors. It is interesting to note that in our series of tumors, the 8p amplicon was always associated with ER-positive status (all 11 cases). Together, these observations strongly suggest that *PPAPDC1B* amplification contributes to oncogenesis through multiple cell signaling pathways. The potential of *PPAPDC1B* as a therapeutic target was further shown by the dramatic inhibition of growth *in vivo* following the silencing of *PPAPDC1B* in breast tumor xenografts displaying 8p11-12 amplification. In this model, *PPAPDC1B* mRNA level at the end of the experiment was about 35% lower after the injection of *PPAPDC1B* siRNAs than after control siRNA injection. This difference, which is significant ($P = 0.0035$), may, however, seem too modest to account for the observed phenotype, but siRNA injections were administered on a weekly basis and inhibition levels were measured 6 days after the last siRNA injection when the effects of the siRNA would be expected to be the weakest. Moreover, the results presented are an

average for different tumor cells and the extinction of *PPAPDC1B* is probably heterogeneous due to differences in the accessibility of different parts of the tumors to siRNA.

A systematic functional study of the genes overexpressed as a consequence of the 8p11-12 amplification in breast tumors was recently published (15). It identified 21 genes for which copy number and expression were strongly correlated. With the exception of *ADAM9*, all the genes identified in this study were also identified by Yang and colleagues. Based on unclearly defined criteria, Yang and colleagues investigated the ability of a subset of 8 of these 21 genes to promote the insulin-independent or epidermal growth factor (EGF)-independent growth of the human mammary MCF10A cell line *in vitro*. *PPAPDC1B, BAG4*, and *LSM1* were included in this panel, but neither *WHSC1L1* nor *DDHD2* was included despite displaying strong correlations between copy number and expression level. Yang and colleagues finally identified *BAG4, LSM1*, and *C8orf4* as breast cancer oncogenes. Thus, two genes, *LSM1* and *BAG4*, were identified in both our study and in that by Yang and colleagues. Unlike us, Yang and colleagues did not identify *PPAPDC1B* as an oncogene. This difference in results may be linked to differences in the functional tests used in the two studies. Indeed, Yang and colleagues assessed the growth factor–independent proliferation of MCF10A cells (on insulin and EGF), whereas we assessed the loss of transforming properties after gene knockdown in 8p-amplified breast cancer cell lines and carried out NIH-3T3 cell transformation assays. The difference in the results obtained in these two studies suggests that *PPAPDC1B* promotes cell transformation without influencing growth factor–dependent cell growth. The role of the various isoforms described for *PPAPDC1B* should also be investigated in this context. PPAPDC1B seems hence to have effects on both cell survival and cell transformation, which could be accounted for by the activation of different signaling pathways, such as the MAPK and JAK-STAT signaling pathways. Such dual effects on cell survival and cell transformation are not unique to *PPAPDC1B* and have already been reported for another gene of the 8p11-12 amplicon, *LSM1*. Indeed, the down-regulation of *LSM1* expression, using siRNA, in the 8p-amplified breast cell line SUM44 results in the inhibition of growth in soft agar (19), and antisense *LSM1* has been shown to decrease cell viability and to increase apoptosis in pancreatic cancer (39).

The only published report on *PPAPDC1B* suggests, based on the down-regulation of this gene in metastatic human hepatocellular carcinoma (HCC) and on experimental criteria, that it may act as a metastasis suppressor in HCC (40). In contrast, its overexpression in a subset of breast cancers due to gene amplification, together with the experimental data reported here, indicates that it may have an opposite, oncogenic role in a different cell background. Alternatively, there may be functional differences between the different *PPAPDC1B* isoforms. Such isoform-specific properties have been described for *BCL2L1*, which encodes both apoptotic, Bcl-X(S), and antiapoptotic, Bcl-X(L), proteins (41).

Yang and colleagues also identified *C8orf4* as a putative oncogene. This gene is located outside the minimal region reported here and was therefore not studied further. However, nonoverlapping minimal regions of amplification have been described in the 8p11-12 region, and it is therefore likely that genes other than *LSM1, BAG4, PPAPDC1B*, and *WHSC1L1*, such as *C8orf4*, also contribute to breast cancer development. The presence of several driver genes is reminiscent of the situation observed for the 17q12 amplicon, in which the overexpression of *STARD3* and *GRB7*, in addition to *ERBB2*, contributes to cell proliferation (6). Moreover, several other regions of amplification within 17q have been described. They may occur in association with or independently of the *ERBB2* amplicon. This report, together with previous publications focusing on the 8p11-12 amplicon, suggests that 8p may display similar variability. Indeed, the 8p amplicon is reported to be large in most cases, encompassing the entire region, and rare amplicons limited to regions proximal (42) or distal (13, 17) to *PPAPDC1B* have been described. One group has also suggested that the 8p11-12 amplicon could be subdivided into different amplicons (14).

We nevertheless believe that, like ERBB2 for the 17q12 amplicon, PPAPDC1B constitutes a major potential therapeutic target in the 8p12 amplicon. Indeed, in addition to the effects of this gene on apoptosis and cloning efficiency *in vitro* and on tumor growth *in vivo, PPAPDC1B* encodes a putative transmembrane phosphatase that may constitute an interesting target for small molecules or antibodies directed against its catalytic and extracellular domains, respectively.

Little is currently known about *PPAPDC1B*. Its identification as a potential therapeutic target in breast cancer highlights the need for further studies investigating its role in cancer, normal development, and tissue homeostasis. It will also be of great interest to assess its potential role in the various cancers harboring an amplification of the 8p11-12 region (43, 44).

Finally, there is growing interest in the potential of phosphatases as drug targets in various diseases, including diabetes, obesity, and cancer (45–47). In cancer, two recent reports have highlighted the role of the PTP1B phosphatase, encoded by a gene in the 20q13 region, which is also amplified in breast cancer, in *ERBB2*-induced mammary tumorigenesis (7, 8). This rising interest in this class of molecules should facilitate the development of specific drugs against *PPAPDC1B*.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Collins C, Volik S, Kowbel D, et al. Comprehensive genome sequence analysis of a breast cancer amplicon. Genome Res 2001;11:1034–42.
2. Hyman E, Kauraniemi P, Hautaniemi S, et al. Impact of DNA amplification on gene expression patterns in breast cancer. Cancer Res 2002;62:6240–5.
3. Al-Kuraya K, Schraml P, Torhorst J, et al. Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 2004;64:8534–40.
4. Ethier SP. Identifying and validating causal genetic alterations in human breast cancer. Breast Cancer Res Treat 2003;78:285–7.

5. de Bono JS, Rowinsky EK. The ErbB receptor family: a therapeutic target for cancer. Trends Mol Med 2002;8: S19–26.
6. Kao J, Pollack JR. RNA interference-based functional dissection of the 17q12 amplicon in breast cancer reveals contribution of coamplified genes. Genes Chromosomes Cancer 2006;45:761–9.
7. Bentires-Alj M, Neel BG. Protein-tyrosine phosphatase 1B is required for HER2/Neu-induced breast cancer. Cancer Res 2007;67:2420–4.
8. Julien SG, Dube N, Read M, et al. Protein tyrosine phosphatase 1B deficiency or inhibition delays ErbB2-induced mammary tumorigenesis and protects from lung metastasis. Nat Genet 2007;39:338–46.
9. Theillet C, Adelaide J, Louason G, et al. FGFRI and PLAT genes and DNA amplification at 8p12 in breast and ovarian cancers. Genes Chromosomes Cancer 1993; 7:219–26.
10. Adelaide J, Chaffanet M, Imbert A, et al. Chromosome region 8p11-p21: refined mapping and molecular alterations in breast cancer. Genes Chromosomes Cancer 1998;22:186–99.
11. Ugolini F, Adelaide J, Charafe-Jauffret E, et al. Differential expression assay of chromosome arm 8p genes identifies Frizzled-related (FRP1/FRZB) and fibroblast growth factor receptor 1 (FGFR1) as candidate breast cancer genes. Oncogene 1999;18:1903–10.
12. Stec I, van Ommen GJ, den Dunnen JT. WHSC1L1, on human chromosome 8p11.2, closely resembles WHSC1 and maps to a duplicated region shared with 4p16.3. Genomics 2001;76:5–8.
13. Garcia MJ, Pole JCM, Chin S-F, et al. A 1 Mb minimal amplicon at 8p11-12 in breast cancer identifies new candidate oncogenes. Oncogene 2005;24:5235–45.
14. Gelsi-Boyer V, Orsetti B, Cervera N, et al. Comprehensive profiling of 8p11-12 amplification in breast cancer. Mol Cancer Res 2005;3:655–67.
15. Yang ZQ, Streicher KL, Ray ME, Abrams J, Ethier SP. Multiple interacting oncogenes on the 8p11-12 amplicon in human breast cancer. Cancer Res 2006;66:11632–43.
16. Ray ME, Yang ZQ, Albertson D, et al. Genomic and expression analysis of the 8p11-12 amplicon in human breast cancer cell lines. Cancer Res 2004;64:40–7.
17. Prentice LM, Shadeo A, Lestou VS, et al. NRG1 gene rearrangements in clinical breast cancer: identification of an adjacent novel amplicon associated with poor prognosis. Oncogene 2005;24:7281–9.
18. Reis-Filho JS, Simpson PT, Turner NC, et al. FGFR1 emerges as a potential therapeutic target for lobular breast carcinomas. Clin Cancer Res 2006;12:6652–62.
19. Streicher KL, Yang ZQ, Draghici S, Ethier SP. Transforming function of the LSM1 oncogene in human breast cancers with the 8p11-12 amplicon. Oncogene 2007;26:2104–14.
20. Chirgwin JM, Przybyla AE, MacDonald RJ, Rutter WJ. Isolation of biologically active ribonucleic acid from sources enriched in ribonuclease. Biochemistry 1979;18: 5294–9.
21. Coombs LM, Pigott D, Proctor A, Eydmann M, Denner J, Knowles MA. Simultaneous isolation of DNA, RNA, and antigenic protein exhibiting kinase activity from small tumor samples using guanidine isothiocyanate. Anal Biochem 1990;188:338–43.
22. Idbaih A, Marie Y, Pierron G, et al. Two types of chromosome 1p losses with opposite significance in gliomas. Ann Neurol 2005;58:483–7.
23. Reyal F, Stransky N, Bernard-Pierrot I, et al. Visualizing chromosomes as transcriptome correlation maps: evidence of chromosomal domains containing co-expressed genes—a study of 130 invasive ductal breast carcinomas. Cancer Res 2005;65:1376–83.
24. Minakuchi Y, Takeshita F, Kosaka N, et al. Atelocollagen-mediated synthetic small interfering RNA delivery for effective gene silencing *in vitro* and *in vivo*. Nucleic Acids Res 2004;32:e109.
25. Blangiardo M, Richardson S. Statistical tools for synthesizing lists of differentially expressed features in related experiments. Genome Biol 2007;8:R54.
26. Milhavet O, Gary DS, Mattson MP. RNA interference in biology and medicine. Pharmacol Rev 2003;55:629–48.
27. Liang Z, Yoon Y, Votaw J, Goodman MM, Williams L, Shim H. Silencing of CXCR4 blocks breast cancer metastasis. Cancer Res 2005;65:967–71.
28. Draghici S, Khatri P, Bhavsar P, Shah A, Krawetz SA, Tainsky MA. Onto-Tools, the toolkit of the modern biologist: Onto-Express, Onto-Compare, Onto-Design and Onto-Translate. Nucleic Acids Res 2003;31:3775–81.
29. Draghici S, Khatri P, Martins RP, Ostermeier GC, Krawetz SA. Global functional profiling of gene expression. Genomics 2003;81:98–104.
30. Draghici S, Khatri P, Shah A, Tainsky MA. Assessing the functional bias of commercial microarrays using the onto-compare database. Biotechniques 2003;Mar, Suppl:55–61.
31. Draghici S, Khatri P, Tarca AL, et al. A systems biology approach for pathway level analysis. Genome Res 2007;17:1537–45.
32. Draghici S, Sellamuthu S, Khatri P. Babel's tower revisited: a universal resource for cross-referencing across annotation databases. Bioinformatics 2006;22: 2934–9.
33. Khatri P, Bhavsar P, Bawa G, Draghici S. Onto-Tools: an ensemble of web-accessible, ontology-based tools for the functional design and interpretation of high-throughput gene expression experiments. Nucleic Acids Res 2004;32:W449–56.
34. Khatri P, Draghici S, Ostermeier GC, Krawetz SA. Profiling gene expression using onto-express. Genomics 2002;79:266–70.
35. Khatri P, Sellamuthu S, Malhotra P, Amin K, Done A, Draghici S. Recent additions and improvements to the Onto-Tools. Nucleic Acids Res 2005;33:W762–5.
36. Still IH, Hamilton M, Vince P, Wolfman A, Cowell JK. Cloning of TACC1, an embryonically expressed, potentially transforming coiled coil containing gene, from the 8p11 breast cancer amplicon. Oncogene 1999;18:4032–8.
37. Tonon G, Wong KK, Maulik G, et al. High-resolution genomic profiles of human lung cancer. Proc Natl Acad Sci U S A 2005;102:9625–30.
38. Sekine Y, Ikeda O, Hayakawa Y, et al. DUSP22/LMW-DSP2 regulates estrogen receptor-α-mediated signaling through dephosphorylation of Ser-118. Oncogene 2007; 26:6038–49.
39. Yan Y, Rubinchik S, Wood AL, et al. Bystander effect contributes to the antitumor efficacy of CaSm antisense gene therapy in a preclinical model of advanced pancreatic cancer. Mol Ther 2006;13:357–65.
40. Wu X, Jia HL, Wang YF, et al. HTPAP gene on chromosome 8p is a candidate metastasis suppressor for human hepatocellular carcinoma. Oncogene 2006;25: 1832–40.
41. Boise LH, Gonzalez-Garcia M, Postema CE, et al. bcl-x, a bcl-2-related gene that functions as a dominant regulator of apoptotic cell death. Cell 1993;74:597–608.
42. Yang ZQ, Albertson D, Ethier SP. Genomic organization of the 8p11-p12 amplicon in three breast cancer cell lines. Cancer Genet Cytogenet 2004;155:57–62.
43. Hurst CD, Fiegler H, Carr P, Williams S, Carter NP, Knowles MA. High-resolution analysis of genomic copy number alterations in bladder cancer by microarray-based comparative genomic hybridization. Oncogene 2004;23:2250–63.
44. Pole JCM, Courtay-Cahen C, Garcia MJ, et al. High-resolution analysis of chromosome rearrangements on 8p in breast, colon and pancreatic cancer reveals a complex pattern of loss, gain and translocation. Oncogene 2006;25:5693–706.
45. Saha S, Bardelli A, Buckhaults P, et al. A phosphatase associated with metastasis of colorectal cancer. Science 2001;294:1343–6.
46. Zhang ZY. Protein tyrosine phosphatases: prospects for therapeutics. Curr Opin Chem Biol 2001;5:416–23.
47. Tonks NK, Muthuswamy SK. A brake becomes an accelerator: PTP1B—a new therapeutic target for breast cancer. Cancer Cell 2007;11:214–6.

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