Comprehensive characterization of the DNA amplification at 13q34 in human breast cancer reveals TFDP1 and CUL4A as likely candidate target genes

Introduction

Breast cancer subtypes exhibit different genomic aberration patterns with a tendency for high-level amplifications in distinct chromosomal regions. These genomic aberrations may drive carcinogenesis through the upregulation of proto-oncogenes. We have characterized DNA amplification at the human chromosomal region 13q34 in breast cancer.

Methods

A set of 414 familial and sporadic breast cancer cases was studied for amplification at region 13q34 by fluorescence in situ hybridization (FISH) analysis on tissue microarrays. Defining the minimal common region of amplification in those cases with amplification at 13q34 was carried out using an array-based comparative genomic hybridization platform. We performed a quantitative real-time polymerase chain reaction (qRT-PCR) gene expression analysis of 11 candidate genes located within the minimal common region of amplification. Protein expression levels of two of these genes (TFDP1 and CUL4A) were assessed by immunohistochemical assays on the same tissue microarrays used for FISH studies, and correlated with the expression of a panel of 33 antibodies previously analyzed.

Results

We have found 13q34 amplification in 4.5% of breast cancer samples, but the frequency increased to 8.1% in BRCA1-associated tumors and to 20% in basal-like tumors. Tumors with 13q34 amplification were associated with high grade, estrogen receptor negativity, and expression of EGFR, CCNE, CK5, and P-Cadherin, among other basal cell markers. We have defined a 1.83 megabases minimal common region of genomic amplification and carried out mRNA expression analyses of candidate genes located therein, identifying CUL4A and TFDP1 as the most likely target genes. Moreover, we have confirmed that tumors with 13q34 amplification significantly overexpress CUL4A and TFDP1 proteins. Tumors overexpressing either CUL4A or TFDP1 were associated with tumor proliferation and cell cycle progression markers.

Conclusions

We conclude that 13q34 amplification may be of relevance in tumor progression of basal-like breast cancers by inducing overexpression of CUL4A and TFDP1, which are both important in cell cycle regulation. Alternatively, as these genes were also overexpressed in non-basal-like tumor samples, they could play a wider role in cancer development by inducing tumor proliferation.
around 5% of breast cancer patients are considered as having hereditary breast cancer. These patients carry mutations in either BRCA1 [1] or BRCA2 [2] genes, but there also are familial breast cancer patients who do not carry mutations in BRCA1/2 and presumably have mutations in another unknown gene or gene(s) (termed non-BRCA1/2 or BRCAX patients). Over the last decade, many studies have shown that sporadic breast cancer can be grouped using molecular profiling into subtypes: basal-like, HER2-overexpressing, luminal A, and luminal B [3-5]. Researchers have identified molecular features that differentiate sporadic breast cancer from each group of familial breast cancers (BRCA1-, BRCA2-, or BRCAX-associated) [6-10]. However, our recent analyses have pointed out the striking similarities between sporadic and familial breast cancer in terms of the existence of breast cancer subtypes in both groups, as well as common patterns of genomic aberrations [11]. This finding may emphasize the interest in identifying molecular features that discriminate each of the sporadic and familial breast cancer subtypes, rather than comparing each group of familial breast cancer with sporadic tumors.

One common genomic aberration in breast cancer is high-level amplification. These aberrations may activate a profound increase in expression of genes within the amplification boundaries, crucial for the origin and progression of breast tumors. The most common regions of high-level amplification in breast cancer are 17q12 (targeting ERBB2), 11q13 (CCND1), 8q24 (MYC); 6p11-p12; 17q22-25; and 20q13 [12,13]. Interestingly, recent genomic analyses have shown that the chromosomal amplification sites tend to differ among the molecular breast cancer subtypes as for instance: 17q12 in HER2-overexpressing tumors, 20q13 in luminal-B tumors, 11q13 in both luminal A and B tumors, or 13q34 in basal-like tumors [5,11,14-16]. The definition of these chromosomal aberrations may elucidate genes crucial for the origin and progression of each breast cancer subtype. Taking this into account, we focused on a comprehensive characterization of 13q34 amplification (Amp13q34), a genomic aberration that we have recently found to be associated with basal-like breast cancers [11]. This amplification has been previously reported in squamous cell carcinomas [17], adrenocortical carcinomas [18], childhood medulloblastoma [19], hepatocellular carcinomas [20], and breast cancer [21], using conventional comparative-genomic hybridization (cCGH). Genes proposed to be the target genes of Amp13q34 include CUL4A, LAMP1, TFDP1, or GAS6 [20,22-26].

We have further characterized Amp13q34 in sporadic and familial breast cancer and defined its overall frequency, as well as its boundaries by fluorescence in situ hybridization (FISH) and array-based comparative genomic hybridization (aCGH) techniques. We have analyzed the gene- and protein-expression levels of candidate genes as well as their correlation with clinical and immunohistochemical (IHC) features. We propose that CUL4A and TFDP1 are likely the driver genes for this genomic amplification, leading higher tumor aggressiveness through deregulation of cell cycle.

Materials and methods

Patients and tumor samples

A total of 188 familial breast cancer patients belonging either to families with at least three women affected with breast and/or ovarian cancer, one of them diagnosed before 50 years of age, or to families with women affected with breast and/or ovarian cancer and at least one case of male breast cancer. All patients were screened for point mutations and large rearrangements in the BRCA1/2 genes using standard methods [27]. A series of 277 sporadic breast cancer samples came from the Spanish National Cancer Center (Spain) (172 samples), the Cancer Center of Solca (Ecuador) (86 cases), and the University of Pennsylvania (USA) (19). Written consent for tumor analyses and publication was obtained from the patient or their relative. This research study has been performed with the approval of an ethics committee.

DNA isolation from formalin-fixed paraffin embedded tumor tissues

Genomic DNA isolation from formalin-fixed paraffin embedded (FFPE) tumors was carried out as previously described [28]. Briefly, two 30-μm sections were obtained from FFPE tumors, treated with xylene, incubated in Glycine Tris-EDTA and NaSCN, and finally digested with proteinase K and purified with phenol chloroform. All sections were previously examined and dissected with a scalpel to ensure at least 70% content of tumor cells.

Array-based comparative genomic hybridization (aCGH)

Comparative genomic hybridization was done onto the 1 megabase (Mb) Bacterial Artificial Chromosome (BAC) clone array platform developed at the University of Pennsylvania. In few words, the platform is composed of 4134 BAC clones spaced at 1 Mb intervals, including a direct coverage of approximately 400 known cancer genes [29]. DNA probe labeling, aCGH protocol, and array data analysis have been described elsewhere [28]. Briefly, normalized aCGH data were analyzed using the Binary Segmentation algorithm implemented in the Insilico CGH software [30]. This algorithm depicts genomic segments showing estimative copy number values in log2 ratio, which are calculated from the mean log2 ratio of all the clones within that segment. As a result, it decreases both the dynamic range of the hybridization and the high noise usually present in hybridizations of DNA from FFPE tissues [31,32]. Therefore, we considered those segments with log2 ratio ≥ 0.1 as gains, whereas those with log2 ratio ≤ -0.1 were categorized as losses. Segments altered with DNA amplifications were considered when log2 ratio ≥ 0.4. A collection of genomic imbalances previously confirmed in breast cancer cell lines and breast tumor samples allowed us to set these thresholds.
Fluorescence in situ hybridization (FISH)

FISH analysis was carried out on the original group of six tumor samples with Amp13q34 found in the aCGH studies to verify their amplification levels. Then, we performed the FISH study on two familial and three sporadic breast cancer tissue microarrays (TMA) containing 156 and 258 breast tumor samples respectively, in order to quantify the Amp13q34 rate in a larger collection. From the familial breast cancer series, 44 samples were already included in our aCGH analysis [28]. These TMA have been described elsewhere [9,33,34]. Briefly, tumor areas of the breast tumor samples were carefully selected from hematoxilin and eosin-stained sections and delimited on the individual paraffin blocks. Then, two tissue cores from the selected tumor area of each specimen were obtained and included in the TMA.

The test FISH probe was composed of three BAC clones mapping to the 13q34 chromosomal region, labeled with dUTP-SpectrumOrange (Vysis, Inc. Downers Grove, IL, USA): RP11-391H12 (AL138221.38, mid-position 113.96 Mb), RP11-102K13 (AL160251.129, 114.09 Mb) and RP11-230F18 (AL442125.13, 114.21 Mb). In addition, three BAC clones from the 13q12.11 chromosomal region were used as copy number reference for chromosome 13, labeled with dUTP-SpectrumGreen (Vysis, Inc. Downers Grove, IL, USA): RP11-301J16 (AL137001.23, 19.63 Mb), RP11-420E5 (AL139327.18, 19.77 Mb), and RP11-385E5 (AL356259.11, 19.92 Mb). FISH analysis was done according to Vysis’ instructions, with slight modifications. An average of 110 (50 to 200) well-defined nuclei was analyzed by scoring the number of single copy 13q34 and 13q12.11 signals. We considered DNA amplification when > 50% of tumor cells showed more than three times as many 13q34 signals as 13q12.11 copy signals.

RNA isolation from formalin-fixed paraffin embedded tissues and cDNA synthesis

Total RNA was obtained from six breast tumors with Amp13q34 (three BRCA1-associated, one BRCA2-associated, one BRCAX-associated, and one sporadic breast tumor samples) and one breast cancer cell line that also carried the amplification (MDA-MB-157); as well as from 13 breast tumors that did not exhibit DNA copy number changes at 13q34. Importantly, 13q34 DNA copy number status for all tumor specimens had been assessed by either aCGH or FISH analyses. All tumor samples were FFPE material, and RNA isolation was carried out as described before [35]. Briefly, a standard tissue sample deparaffinization was performed using xylene and alcohols. Then, samples were incubated in a digestion buffer (10 mM TrisHCl pH 8.0; 0.1 mM EDTA; 2% SDS and 500 μg/ml protease K). Nucleic acids were purified with standard phenol-Chloroform-isomyl alcohol procedures, followed by a precipitation with isopropanol, glycogen and sodium acetate. After DNase treatment (Ambion Inc., Austin, TX, USA) at 37°C for 30 minutes, RNA was finally processed for cDNA synthesis using M-MLV retrotranscriptase enzyme (Invitrogen, Carlsbad, CA, USA).

Quantitative real time polymerase chain reaction (qRT-PCR) for mRNA expression

Assays were designed using the Roche Applied Science Universal Probe Library website [36] (Roche Diagnostics, Basel, Switzerland) for all target genes and endogenous control (see Additional file 1). qRT-PCR assays were set up in triplicates and performed using the ABI Prism 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Relative expression was determined using the free access software qBase [37] based on a modification of the classic delta-delta Ct method that allows for PCR efficiency correction. Appropriate positive and negative controls including non-retrotranscribed RNA for each sample were run for the experiment.

Histology and immunohistochemistry

To associate genomic copy number aberration at 13q34 with changes in protein levels of the candidate genes, we carried out antibody specific staining on the same TMA used in the FISH analyses.

IHC assays were performed by the Envision method (Dako, Glostrup, Denmark) with a heat-induced antigen retrieval step. TMA sections were immersed in 10 mM boiling sodium citrate at pH 6.5 for two minutes in a pressure cooker. Between 150 and 200 cells per core were scored to determine the percentage of cells with positive nuclei or cytoplasm, depending upon the marker. We evaluated the protein expression level for the two candidate genes within Amp13q34 that were significantly overexpressed in the qRT-PCR analyses: CUL4A and TFDP1. As CUL4A expression in the cytoplasm was found in most of cases, we delineated four levels of staining: 0 (null staining), 1 (low staining), 2 (medium staining), and 3 (strong staining), which we grouped into negative (0), mid-level (1, 2) and positive (3) in subsequent analyses. TFDP1 is localized in the cell nucleus, so the median percentage of positive nuclei in all cases was used as the threshold to discriminate between negative (< 25%) or positive expression (≥ 25%). Tumor samples with more than 60% of stained cells (the median percentage of stained cells in only positive cases) were further categorized as highly expressed for the association with Amp13q34.

Other proteins also were studied to establish potential associations. We had previously evaluated nuclear staining for estrogen receptor (ER), progesterone receptor (PR), p53, Ki-67, cyclins D1, D3, E, and A; p16, p27, p21, CDK1, CDK2, CDK4, Skp2, retinoblastoma protein (Rb), E2F1, E2F6, MDM2, topoisomerase IIα, survivin, and CHEK2; cytoplasmic staining for BCL2, vimentin, cytokertatin 5/6 (CK5/6), cytokerin 8 (CK8), and cyclin B1; and membrane staining for E-cadherin, P-cadherin, B-catenin and G-catenin [34]. HER-2 expression was evaluated according to the four-category (0 to
3+) DAKO system proposed for the evaluation of the Herceptest, and HER-2 expression of 3+ was the only value considered positive [34]. Antibodies, dilutions, suppliers, and thresholds used for analyses are listed in Additional file 2.

Statistical analyses

In order to compare the mRNA expression levels between the different groups (tumors with or without Amp13q34), we applied the U-Mann Whitney test. We also determined associations between tumor groups with both clinical features and IHC markers using the chi square test, with the two-tailed Fisher's exact test correction when needed. The statistical software SPSS for Windows (SPSS, Inc., Chicago, IL, USA) was used to perform these statistical comparisons.

Results

Frequency and genomic definition of the 13q34 amplification

We have previously reported that about 5% of both familial and sporadic breast cancers contain a high-level amplification at 13q34 (Amp13q34) using chromosomal- and array-based comparative genomic hybridization techniques (cCGH, and aCGH, respectively) (Table 1 and Figure 1a) [21,28]. Now, we have extended the study of the rate of Amp13q34 to a larger cohort of 414 familial and sporadic breast cancer samples using FISH technique. We have found that Amp13q34 is present in around 4.5% of breast cancer samples (Figure 1b-c), although the rate differs slightly among tumor classes, being higher in BRCA1- (8.1%) than in BRCA2- or non-BRCA1/2- (<3.0%) associated cancers (Table 1). However, these differences are not statistically significant, maybe due to the low number of cases (data not shown). Noteworthy, the level of amplification in this tumor cohort never exceeded a 13q34:13q12.11 copy ratio of seven, being the median ratio of 4.5 as many 13q34 as 13q12.11 copy signals (Figure 1c). This shows that 13q34 region, albeit that it is affected with amplification, is not altered with the same magnitude as other classical high-level DNA amplification sites, such as 17q12 (ERBB2). As expected, no tumor sample analyzed by both cCGH/aCGH and FISH showed discrepancies in copy number values.

To define the minimal common region of amplification, we used data from a previous aCGH analysis [28]. A genomic representation of those cases with Amp13q34 (three BRCA1-, one BRCA2-, one BRCAX-, one sporadic-breast cancers and one breast cancer cell line, MDA-MB-157) is shown in Figure 2a. Chromosome 13 has two different genomic profiles based on the start position of the genomic gain: a) loss from the centromere to the 13q31 or 13q32 chromosomal band, with gain or amplification extending from 13q31 to the telomere (cases 1, 3, 4, 6, and 7); or b) copy neutral from the centromere to 13q21 and gain or amplification from 13q21 to the telomere (cases 2 and 5). A detailed analysis of the genomic values on each of the studied cases allowed us to narrow down the minimal common region of DNA amplification to 1.83 Mb, mapping entirely within the 13q34 chromosomal band. Two samples delimited the Amp13q34 region: tumor #3 showed the Amp13q34 starting at RP11-520D2 (spanning 3.55 Mb, and 29 genes), whereas tumor #5 finally defined the minimal 1.83 Mb region from RP11-375A8 to the telomere that contains 22 genes (Figure 2b) (Table 2). As tumor material was limited to carry out a whole characterization of all the genes therein located, we selected those related to tumorigenesis or cell transformation according to either previous studies or their hypothetical function. Our final list of selected genes included: ARHGEF7, ATP11A, MCF2L, CUL4A, LAMP1, GRTP1, DCUN1D2, TFDP1, GAS6, RASA3, and CDC16 (bold genes in Table 2). Although ARHGEF7 is not located within the boundaries of the 1.83 Mb region, it was selected to check a possible correlation for those genes located outside the minimal region, but inside the
3.55 Mb defined by the case #3. Noteworthy, the 13q34 FISH probe used in this study covered CUL4A, LAMP1, GRTP1, DCUN1D2, and TFDP1 (Figure 2 and Table 2).

**Analyses of the mRNA expression level for the candidate genes by qRT-PCR**

Once the minimal amplification site had been delineated, qRT-PCR was carried out to determine the mRNA expression level of each of the 11 candidate genes, comparing two cohorts of FFPE samples: tumors with Amp13q34 (six tumor samples and MDA-MB-157), and tumors without any genomic aberration at 13q34 region (13 cases). ARHGEF7 and ATP11A showed a non-statistically significant trend to be overexpressed in Amp13q34 tumors (P = 0.242 and P = 0.191, respectively); whereas GAS6 and RASA3 had non-significant tendencies to be downregulated in tumors with Amp13q34 (P = 0.285 and P = 0.052, respectively) (Figure 3). Nevertheless, two genes had significant association between amplification and overexpression: CUL4A (P = 0.007) and TFDP1 (P = 0.019) (Figure 3). At this point, we focused on these two genes as candidate drivers of the 13q34 amplification.

**Correlation with protein expression levels and other immunohistochemical features**

We performed immunohistochemical analyses of the two candidate genes (CUL4A and TFDP1) on a set of 414 breast cancer samples on TMAs, previously analyzed by FISH (see above). Interestingly, the 75% of the tumors with Amp13q34 showed a strong staining for CUL4A, compared with 34.3% of the tumors without Amp13q34, but overexpressing CUL4A (P = 0.010) (Figure 4a-c and Table 3). A significant association was also found between Amp13q34 and TFDP1, since the 92.3% of the tumors with Amp13q34 (12/13) presented TFDP1 overexpression (P = 0.004), whereas the 51.9% of the tumors without Amp13q34 overexpressed TFDP1 (Figure 4d-f and Table 3). Furthermore, when considering a higher percentage (> 60%) of TFDP1 positive cells as the threshold, 76.9% of Amp13q34 tumors had TFDP1 overexpression as compared with 20.1% of the tumors without Amp13q34 (P = < 0.001) (Table 3). Interestingly, 60% of tumors with Amp13q34 co-overexpressed both CUL4A and TFDP1 (6 out of 10 tumors), although it was not statistically significant maybe due to the low number of samples (data not shown). Altogether, these findings demonstrated that Amp13q34 was significantly associated with a higher expression level of both CUL4A and TFDP1, but given that there are cases without the amplification showing protein overexpression, Amp13q34 was not the exclusive mechanism causing overexpression of these genes.

In addition, we associated Amp13q34 with other immunohistochemical and clinical tumor features previously analyzed on a subset of the sample set (260 out of 414 samples) [33,34]. We observed that tumors with Amp13q34 were characterized by high histological grade; an absence of expression of ER, PR, CCND1, RB, p16, and CK8; and an overexpression of Ki-67, EGFR, P-Cadherin, G-catenin, CCNE, CCNB1, SKP2, survivin, vimentin, and CK5 (Table 4). As most of these markers are associated with basal-like phenotype of breast cancer, these associations may be due to the unique presence of the Amp13q34 in basal-like tumors as we previously reported [11]. CUL4A-overexpressing tumors had higher grade, absence of expression of PR and BCL2, and a higher percentage of cases expressing EGFR, P-Cadherin, CCNB1, SKP2, and CK5; when compared with tumors that did not overexpress CUL4A (Table 4). Last, TFDP1-overexpressing tumors were associated with overexpression of CCNE, CCNB1, and p16 (Table 4). However, no significant association was found between TFDP1 and CUL4A expression levels when studying the overall breast cancer sample set (data not shown). These associations support that the overexpression of CUL4A and TFDP1, even though it is correlated with Amp13q34, is not exclusive of basal-like tumors, but still relates to higher tumor aggressiveness.
Discussion

In the present study, we have characterized the genomic amplification at the human 13q34 chromosomal region in familial and sporadic breast cancer cases. We have defined a minimal common region of amplification using FISH and aCGH techniques. Moreover, we have characterized the gene and protein expression levels of eleven candidate genes within the region using qRT-PCR and immunohistochemistry assays. The correlations that we have found suggest TFDP1 and CUL4A as likely drivers of this genomic aberration.

Amp13q34 previously has been reported in breast cancer [22,23] as well as in squamous cell carcinomas, adrenocortical carcinomas, childhood medulloblastoma, and hepatocellular carcinomas [17-20]. In our analyses, Amp13q34 is a low frequent genomic event in overall breast cancer (around 4.5%) but the rate increases when analyzing BRCA1-associated breast cancer (8.1%) (Table 1) or basal-like tumors (about 20%) [11]. Although this increase is not statistically significant (data not shown), it is in agreement with previous analyses that describe genomic gains/amplifications at 13q34 in ER-nega-
| Gene Name* | Reference Sequence | Chromosome location (start pb - end pb)*** | Name | Additional information |
|------------|-------------------|--------------------------------------------|------|------------------------|
| ANKRD10    | NM 017664         | 111530888-111567416                        | ankyrin repeat domain 10 |                        |
| ARHGEF7    | NM 145735.2       | 111767624-111947542                        | Rho guanine nucleotide exchange factor (GEF) 7 | Rho GTPase |
| C13orf16   | NM 152324.1       | 111973015-111996593                        | chromosome 13 open reading frame 16 |                        |
| SOX1       | NM 005986.2       | 112721913-112726020                       | SRY (sex determining region Y)-box 1 | Transcription factor involved in the regulation of embryonic development and in the determination of the cell fate |
| C13orf28   | NM 145248.3       | 113030669-113089001                       | chromosome 13 open reading frame 28 |                        |
| TUBGCP3    | NM 006322.4       | 113139328-113242481                       | tubulin, gamma complex associated protein 3 |                        |
| C13orf35   | NM 207440.1       | 113301358-113338811                       | chromosome 13 open reading frame 35 |                        |
| ATP11A     | NM 032189.3       | 113344643-113541480                       | ATPase, class VI, type 11A | Integral membrane ATPase |
| MCF2L      | NM 001112732.1    | 113622757-113752862                       | MCF2 cell line derived transforming sequence-like |                        |
| F7         | NM 000131.3       | 113760105-113774994                       | coagulation factor VII (serum prothrombin conversion accelerator) | Coagulation factor. Defects in this gene can cause coagulopathy |
| F10        | NM 000504.3       | 113777113-113803841                       | coagulation factor X | Coagulation factor. Mutations of this gene result in factor X deficiency, a hemorrhagic condition of variable severity. |
| PROZ       | NM 003891.1       | 113812968-113826694                       | protein Z, vitamin K-dependent plasma glycoprotein |                        |
| PCID2      | NM 018386.2       | 113831925-113863029                       | PCI domain containing 2 |                        |
| CUL4A      | NM 001008895.1    | 113863931-113919391                       | culin 4A |                        |
| LAMP1      | NM 005561.3       | 113951469-113977741                       | lysosomal-associated membrane protein 1 | Membrane glycoprotein. It may also play a role in tumor cell metastasis |
| GRTP1      | NM 024719.2       | 113978506-114018463                       | growth hormone regulated TBC protein 1 |                        |
| ADPRHL1    | NM 138430.3       | 114076586-114107839                       | ADP-ribosylhydrolase like 1 | Reversible posttranslational modification used to regulate protein function |
| DCUN1D2    | NM 001014283.1    | 114110134-114145023                       | DCN1, defective in cullin neddylation 1, domain containing 2 (S. cerevisiae) |                        |
| TMCO3      | NM 017905.4       | 114145308-114204542                       | transmembrane and coiled-coil domains 3 |                        |
| TFDP1      | NM 007111.4       | 114239056-114295786                       | transcription factor Dp-1 | Transcription factor that heterodimerizes with E2F proteins to enhance their DNA-binding activity and promote transcription from E2F target genes. |
| ATP4B      | NM 000705.2       | 114303123-114312501                       | ATPase, H+/K+ exchanging, beta polypeptide | Encodes the beta subunit of the gastric H+, K+-ATPase |
| GRK1       | NM 002929.2       | 114321597-114438636                       | G protein-coupled receptor kinase 1 | Ser/Thr protein kinase that phosphorylates rhodopsin and initiates its deactivation. Defects in GRK1 are known to cause Oguchi disease 2 |
tive tumors, basal-like tumors, BRCA1-associated breast cancers, and medullary carcinomas [10,16,38,39]. Furthermore, we found that Amp13q34 is associated with a high histological grade, hormonal receptor negativity, and an overexpression of basal cytokeratins, cell cycle promoters (CCNE, CCNB1) and EGFR, among other markers (Table 4). These findings suggest that Amp13q34 is biologically important in the progression of basal-like breast cancers. Although a screening in a larger basal-like tumor cohort would be needed; once the prevalence of Amp13q34 is confirmed in this breast tumor phenotype, this genomic aberration could be used both as a clinical marker and as a therapeutic target for Amp13q34 tumors.

As far as we know, this is the first high-resolution genomic characterization of the amplification at the human 13q34 chromosomal site. We narrowed down the minimal common region of Amp13q34 to 1.83 Mb, which included 22 genes, of which 11 may be related to tumorigenesis: ARHGEF7, ATP11A, MCP2L, CUL4A, LAMP1, GRTP1, DCUN1D2, TFDP1, GAS6, RASA3, and CDC16 (Figure 2 and Table 2). In a recent study of mammary tumors arising in a p53-null mouse model, a genomic amplification at mouse chromosomal 8A1 region, syntenic to the human 13q34 region, was defined with a high-resolutive genomic BAC array. The authors reported two minimal clusters of amplification that also covered the aforementioned genes. In addition, they described amplification of CUL4A (25.7%), LAMP1 (13.5%), TFDP1 (31.1%), and GAS6 (13.5%) by DNA qRT-PCR on a set of 74 human breast carcinomas [22]. Notably, the amplification and overexpression of CUL4A was also reported in primary breast cancers a few years ago [23]. All these findings support a role for Amp13q34 in human breast cancer, likely through the increased expression of the genes located therein.

Based on the correlations observed in this study, CUL4A and TFDP1 are suggested to be the target genes for this genomic amplification. Both their mRNA (Figure 3) and proteins (Figure 4, and Table 3) are significantly overexpressed in breast tumors with Amp13q34 when compared with breast tumors without 13q34 genomic alterations. Therefore, our data support the importance of these genes as targets for the amplification, consistent with previous findings not only in breast cancer [22], but also in hepatocellular carcinomas [20].

CUL4A is a member of the cullin protein family and composes the multifunctional ubiquitin-protein ligase E3 complex [40]. Our results suggest that CUL4A may play a role in breast can-
Figure 3

Gene expression analysis by qRT-PCR of the eleven candidate genes located in the minimal common regions of Amp13q34. Relative expression levels of the candidate genes in two sample cohorts: breast tumors with no genomic aberration at 13q34 (grey), and breast tumors with Amp13q34 (black). The expression of the candidate genes was evaluated by real-time quantitative RT-PCR and normalized with a housekeeping gene (ACTB). P values are shown for those genes with statistically significant differences when comparing the two groups in a U-Mann Whitney Test (P < 0.05).

Figure 4

Immunohistochemical staining for CUL4A (a-c) and TFDP1 (d-f) in tumors with and without Amp13q34. (a) Null CUL4A staining in a non-amplifier tumor sample. (b) Moderate CUL4A staining in a non-amplifier tumor sample. (c) Strong CUL4A staining in a tumor with the Amp13q34. (d) Negative TFDP1 expression in a tumor without Amp13q34. (e) and (f) Strong TFDP1 expression in tumors with Amp13q34, the arrow in (f) points out the absence of expression in a lymphocyte infiltrate compared with the breast tumor cells.
E2F1 expression suggests that TFDP1 may have a function in
(data not shown). The lack of correlation between TFDP1 and
one of the E2F1/TFDP1 regulators, RB, in our tumor cohort
altered expression levels either of its protein partner, E2F1, or
However, overexpression of TFDP1 did not correlate with
tions point out a deregulation of the cell cycle in these tumors.


described in hepatocellular carcinoma [20]. These associa-
tion of cell cycle promoters (cyclin A, cyclin E, CDK2) [45]. In
our study, breast tumors overexpressing TFDP1 had high
expression of p16, cyclin E and cyclin B1 (Table 4), as
documented in breast malignancies [22,23], as well as in other carcinomas [20]. It has been
shown that CUL4A could contribute to tumor malignancy
mediating the activity of tumor suppressors and thus, altering
cell cycle checkpoints [41-44]. Moreover, a recent study has
reported that breast cancer patients with strong expression of
CUL4 had a significantly shorter overall and disease-free sur-
vival [25]. In our analysis, CUL4A overexpressing tumors were
characterized by a high histological grade, an absence of PR
and BCL2 expression, and a high expression of cell cycle pro-
motors (cyclin B and SKP2), cell signaling components
(EGFR), basal cytoskeleton (CK5), and cell adhesion proteins
(P-cadherin) (Table 4), suggesting high cell proliferation and
tumor aggressiveness. As some of these features are basal-
cell markers (such as EGFR, CK5, P-Cadherin), CUL4A may
play an important role in the biology of basal-like breast can-
cers. Furthermore, our results suggest the role for CUL4A as
part of the development of breast tumors in general, mainly
through deregulation of cell cycle checkpoints.

TFDP1 is a heterodimerization partner for members of the E2F
family of transcription factors. E2F1/TFDP1 forms a complex
involved in cell cycle progression by the regulation of expres-
sion of cell cycle promoters (cyclin A, cyclin E, CDK2) [45]. In
our study, breast tumors overexpressing TFDP1 had high
expression of p16, cyclin E and cyclin B1 (Table 4), as
described in hepatocellular carcinoma [20]. These associations
point out a deregulation of the cell cycle in these tumors.
However, overexpression of TFDP1 did not correlate with altered expression levels either of its protein partner, E2F1, or
one of the E2F1/TFDP1 regulators, RB, in our tumor cohort
(data not shown). The lack of correlation between TFDP1 and
E2F1 expression suggests that TFDP1 may have a function in
addition to gene transcription regulation and cell cycle pro-
gression in breast cancer. This discordance between E2F1 and
TFDP1 protein levels was also described in non-Hodgkin
lymphomas [46] and in hepatocellular carcinomas, where only
TFDP1 overexpression was associated with a larger tumor
size [26]. Additionally, Abba and colleagues analyzed publicly
available human breast cancer mRNA expression datasets
and found significant associations of TFDP1 overexpression
with shorter overall survival, relapse-free survival, and metastasis-free interval [22]. Moreover, TFDP1 overexpression has
been related to progression of hepatocellular carcinomas [26],
and together with activated HA-RAS, causes oncogenesis in
rat embryo fibroblasts [47]. Thus, TFDP1 is not only a strong
candidate to drive Amp13q34, but also a gene involved in dif-
f erent components of tumorigenesis, causing higher tumor
aggressiveness and a poor patient prognosis. Further func-
tional analyses to elucidate its potential role in breast cancer
oncogenesis need to be performed.

The other candidate genes that we studied (ARHGEF7, ATP11A, MCF2L, LAMP1, GRTP1, DCUN1D2, GAS6, RASA3 and CDC16) did not demonstrate overexpression in
Amp13q34 tumors, although non-significant trends were
found for overexpression of ARHGEF7 and ATP11A, and
downregulation of GAS6 and RASA3 (Figure 3). However,
the lack of significant correlations for these genes between
copy number and expression in our dataset should not rule out
their possible role in breast cancer. For example, a recent arti-
cle associated expression of GAS6 with indicators of good
prognosis such as progesterone receptor positivity, small
tumor size, low grade, and young patient age [24]. These
tumor features do not correspond to the ones found in
Amp13q34 tumors, which are mainly ER and PR negative
(Table 4); thus, GAS6 may not be the target gene in the
Amp13q34, but may still play a role in other breast cancers.

| Amp     | No amp | No amp | No amp |
|---------|--------|--------|--------|
|         | 155 (65.7) | 81 (34.3) | 4 (17.1) |
|         | 151 (64.0) | 81 (34.3) | 4 (1.7) |
|         | 115 (48.1) | 124 (51.9) | 1 (7.7) |
|         | 1 (7.7) | 12 (42.3) | 1 (7.7) |
|         | 1 (7.7) | 12 (42.3) | 1 (7.7) |
|         | 12 (42.3) | 10 (76.9) | 12 (42.3) |

P-values: P = 0.010 * P = 0.004 * P = 0.001 *
### Table 4

Protein marker associations between tumors with/without Amp13q34, CUL4A expression, or TFDP1 expression

| Clinical & IHC feature | 13q34 Amplification | CUL4A expression | TFDP1 expression |
|------------------------|----------------------|------------------|------------------|
|                        | Absence  | Presence  |  P             | Negative | Positive |  P             | Negative | Positive |  P             |
| **Grade**              |          |          |               |          |          |               |          |          |               |
| 1                      | 42 (32.1)| 0        | 0.002*        | 26 (40.6)| 10 (19.2)| 0.010*       | 12 (32.4)| 27 (33.3)| NS*           |
| 2                      | 40 (30.5)| 0        |               | 19 (29.7)| 13 (25.0)|             | 11 (29.7)| 22 (27.2)|               |
| 3                      | 49 (37.4)| 8 (100)  |               | 19 (29.7)| 29 (55.8)|             | 14 (37.8)| 32 (39.5)|               |
| **ER**                 |          |          |               |          |          |               |          |          |               |
| Negative               | 56 (35.4)| 8 (88.9)| 0.002         | 30 (36.6)| 29 (48.3)| NS*          | 24 (46.2)| 35 (38.0)| NS            |
| Positive               | 102 (64.6)| 1 (11.1)|              | 52 (63.4)| 31 (51.7)|             | 28 (53.8)| 57 (62.0)|               |
| **PR**                 |          |          |               |          |          |               |          |          |               |
| Negative               | 68 (47.2)| 8 (88.9)| 0.018         | 30 (43.5)| 37 (62.7)| 0.034        | 20 (48.8)| 47 (52.3)| NS            |
| Positive               | 76 (52.8)| 1 (11.1)|              | 39 (56.5)| 22 (37.3)|             | 21 (51.2)| 42 (47.7)|               |
| **BCL2**               |          |          |               |          |          |               |          |          |               |
| Negative               | 82 (56.9)| 8 (88.9)| 0.082         | 37 (53.6)| 42 (71.2)| 0.047        | 29 (70.7)| 52 (58.4)| NS            |
| Positive               | 62 (43.1)| 1 (11.1)|              | 32 (46.4)| 17 (28.8)|             | 12 (29.3)| 37 (41.6)|               |
| **Ki-67**              |          |          |               |          |          |               |          |          |               |
| 0-5%                   | 68 (47.2)| 1 (11.1)| <0.001*       | 35 (50.7)| 22 (37.3)| NS*          | 22 (53.7)| 35 (39.3)| NS            |
| 6-25%                  | 56 (38.9)| 1 (11.1)|              | 24 (34.8)| 20 (33.9)|             | 13 (31.7)| 32 (36.0)|               |
| > 25%                  | 20 (13.9)| 7 (77.8)|              | 10 (14.5)| 17 (28.8)|             | 6 (14.6)| 22 (24.7)|               |
| **EGFR**               |          |          |               |          |          |               |          |          |               |
| Negative               | 181 (90.0)| 4 (40.0)| <0.001        | 122 (91.7)| 57 (80.3)| 0.024        | 94 (91.3)| 89 (85.6)| NS            |
| Positive               | 20 (10.0)| 6 (60.0)|              | 11 (8.3)| 14 (19.7)|             | 9 (8.7)| 15 (14.4)|               |
| **Cadherin P**         |          |          |               |          |          |               |          |          |               |
| Negative               | 128 (92.1)| 3 (33.3)| <0.001        | 63 (95.5)| 46 (79.3)| 0.011        | 37 (94.9)| 74 (86.0)| NS            |
| Positive               | 11 (7.9)| 6 (66.6)|              | 3 (4.5)| 12 (20.7)|             | 2 (5.1)| 12 (14.0)|               |
| **G-Catenin**          |          |          |               |          |          |               |          |          |               |
| Negative               | 106 (79.1)| 4 (44.4)| 0.031         | 59 (86.8)| 43 (75.4)| NS           | 36 (90.0)| 69 (78.4)| NS            |
| Positive               | 28 (20.9)| 5 (55.5)|              | 9 (13.2)| 14 (24.6)|             | 4 (10.0)| 19 (21.6)|               |
| **Cyclin D1**          |          |          |               |          |          |               |          |          |               |
| Negative               | 67 (47.2)| 8 (88.9)| 0.018         | 32 (47.1)| 27 (46.6)| NS           | 17 (42.5)| 42 (47.7)| NS            |
| Positive               | 75 (52.8)| 1 (11.1)|              | 36 (52.9)| 31 (53.4)|             | 23 (57.5)| 46 (52.3)|               |
| **Cyclin E**           |          |          |               |          |          |               |          |          |               |
| Negative               | 103 (72.5)| 2 (22.2)| 0.004         | 50 (73.5)| 34 (58.6)| 0.090        | 32 (80.0)| 53 (60.2)| 0.043         |
| Positive               | 39 (27.5)| 7 (77.8)|              | 18 (26.5)| 24 (41.4)|             | 8 (20.0)| 35 (39.8)|               |
| **Cyclin B1**          |          |          |               |          |          |               |          |          |               |
| Negative               | 111 (80.4)| 4 (44.4)| 0.024         | 57 (86.4)| 40 (70.2)| 0.045        | 37 (92.5)| 61 (72.6)| 0.010         |
| Positive               | 27 (19.6)| 5 (55.6)|              | 9 (13.6)| 17 (29.8)|             | 3 (7.5)| 23 (27.4)|               |
Conclusions

In summary, we have characterized a 1.83 Mb minimal common region of amplification at 13q34 that may play a crucial role in the development of basal-like breast cancers. Analyses of larger series of this tumor subtype will allow us to confirm the specificity of this aberration and, importantly, its possible use as a clinical marker of basal-like tumors. This genomic aberration could facilitate the tumor progression through the overexpression of driver/target genes such as TFDP1 and CUL4A, which are overexpressed not only in Amp13q34 tumors, but also in other breast cancer samples characterized by a high cell proliferation and aggressiveness. Therefore, further functional analyses should be performed to demonstrate their potential oncogenic roles, so that the development of pharmaceutical suppressors of CUL4A or TFDP1 activity could provide a therapeutic target for tumors overexpressing these proteins.

Competing interests

The authors declare that they have no competing interests.

Authors’ contributions

LM carried out the genomic and qRT-PCR analyses, performed the statistical analyses and drafted the manuscript. LPSC carried out the qRT-PCR assays. Pathologists IMR, SMRP, EH and JP analyzed the immunoassays. AC and JP provided tumor sample sets for the study. KLN provided the array-CGH platform and helped to draft the manuscript. MJG
participated in the design of the study. JB conceived of the study, participated in its design and coordination, and helped to draft the manuscript. All authors read and approved the final manuscript.

Additional files

The following Additional files are available online:

Additional file 1
Word document containing a table showing genes, primers and Universal Probe Library from Roche® used for qRT-PCR analyses in the present study. See http://www.biomedcentral.com/content/supplementary/bcr2456-S1.doc

Additional file 2
Word file containing a table listing the antibodies used in the present immunohistochemical analysis and thresholds established to consider a tumor as positive. See http://www.biomedcentral.com/content/supplementary/bcr2456-S2.doc

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References

1. Miki Y, Swensen J, Shattuck-Eidens D, Futreal PA, Harshman K, Tavtigian S, Liu Q, Cochran C, Bennett LM, Ding W, et al.: A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. Science 1994, 266:67-71.

2. Wooster R, Neuhausen SL, Mangion J, Quirk Y, Ford D, Collins N, Nguyen K, Seal S, Tran T, Averill D, et al.: Localization of a breast cancer susceptibility gene, BRCA2, to chromosome 13q12-13. Science 1994, 265:2508-2509.

3. Hu Z, Fan C, Oh DS, Marron JS, He X, Qaqish BF, Livasy C, Carey LA, Reynolds E, Dressler L, Noel A, Parker J, Eweng MG, Sawyer LR, Wu J, Liu Y, Nanda R, Tretiakova M, Ruiz Omrco A, Dreher D, Palazzo JP, Perreard L, Nelson E, Mone M, Hansen H, Mullins M, Quackenbush JF, Ellis MJ, Olopade OI, Bernard PS, Perou CM: The molecular portraits of breast tumors are conserved across microarray platforms. BMC Genomics 2006, 7:86.

4. Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA, Fluge O, Pergamenschikov A, Williams C, Zhu SX, Lonning PE, Borresen-Dale AL, Brown PO, Botstein D: Molecular portraits of human tumors. Nature 2000, 406:747-752.

5. Bozamachis A, Kim YH, Wang P, Goris TL, Hernandez-Boussard T, Lonning PE, Tibshirani R, Borresen-Dale AL, Pollack JR: Distinct patterns of DNA copy number alteration are associated with different clinicopathological features and gene-expression subtypes of breast cancer. Genes Chromosomes Cancer 2006, 45:1033-1040.

6. Hedenfalk I, Duggan D, Chen Y, Radmacher M, Bittner M, Simon R, Meltzer P, Gusterson B, Esteller M, Kallioniemi OP, Wilfond B, Aittomaki K, Sauter G: Gene-expression profiles in hereditary breast cancer. N Engl J Med 2001, 344:539-548.

7. Jonsson G, Naylor TL, Vallon-Christersson J, Stafa J, Huang J, Ward MR, Greshock JD, Luts L, Olsson H, Rahman N, Stratton M, Ringner M, Borg A, Weber BL: Distinct genomic profiles in hereditary breast tumors identified by array-based comparative genomic hybridization. Cancer Res 2005, 65:7612-7621.

8. Lakhani SR, Vijver MJ, Van De, Jacquemier J, Anderson TJ, Oesin PP, Muffo L, Easton DF, Theogf R: Pathology of familial breast cancer: predictive value of immunohistochemical markers estrogen receptor, progesterone receptor, HER-2, and p53 in patients with mutations in BRCA1 and BRCA2. J Clin Oncol 2002, 20:2310-2318.

9. Palacios J, Honrado E, Osorio A, Cazoria A, Sarrio D, Barroso A, Rodriguez S, Cigudosa JC, Diez O, Alonso C, Lerma E, Sanchez R, Rivas C, Benitez J: Immunohistochemical characteristics defined by tissue microarray of hereditary breast cancer not attributable to BRCA1 or BRCA2 mutations: differences from breast carcinomas arising in BRCA1 and BRCA2 mutation carriers. Clin Cancer Res 2003, 9:3606-3614.

10. Joosse SA, van Beers EH, Tielen IH, Horlings H, Peterse JL, Hoogerbrugge N, Liigtenberg MJ, Wessels LF, Aqwisk P, Verhoef S, Hogevorst FB, Nederlof PM: Prediction of BRCA1-association in hereditary non-BRCA1/2 breast carcinomas with array-CGH. Breast Cancer Res Treat 2008.

11. Melchor L, Honrado E, Garcia MJ, Alvarez S, Palacios J, Osorio A, Nathanson KL, Benitez J: Distinct genomic aberration patterns are found in familial breast cancer associated with different immunohistochemical subtypes. Oncogene 2008, 27:3165-3175.

12. Al-Kuraya K, Schraml P, Torhorst J, Tapia C, Zaharieva B, Novotny H, Spichitin H, Maurer R, Mrlacher M, Kochl O, Zuber M, Dietenrich H, Mross F, Wilber K, Schol R, Sabatier G: Prognostic relevance of gene amplifications and coamplifications in breast cancer. Cancer Res 2004, 64:8534-8540.

13. Albertson DG, Sijnders AM, Fridyland J, Jordan R, Pinkel D, Schmidt BL: Genomic analysis of tumors by array comparative genomic hybridization: more is better. Cancer Res 2006, 66:3955-3956. author reply 3956.

14. Adelaide J, Finetti P, Bekhouche I, Repellini L, Geneix J, Sircou- lomb F, Charafe-Jauffret E, Cervera N, Desplans P, Parzy D, Schoenmakers E, Viens P, Jacquemier J, Bimbamud B, Bertucci F, Chaffanet M: Integrated profiling of basal and luminal breast cancers. Cancer Res 2007, 67:11565-11575.

15. Chin K, DeVries S, Fridyland J, Spellman PT, Roysagupta R, Kuo WL, Lapuk A, Neve RM, Qian Z, Ryder T, Chen F, Feiler H, Toku- yasu T, Kingsley C, Dairke S, Meng Z, Chew K, Pinkel D, Jain A, Luqun BM, Esserman L, Bertucci F, Widmat FM, Gasy JW: Genomic and transcriptional aberrations linked to breast cancer pathophysiology. Cancer Cell 2006, 10:529-541.

16. Natrajan R, Lambros MB, Rodriguez-Pinilla SM, Moreno-Bueno G, Tan DS, Marchio C, Vatcheva R, Rayter S, Mahler-Araujo B, Fulford LG, Hungermann D, Mackay A, Grigoriadis A, Feniak K, Tamber N, Hardisson D, Tutt A, Palacios J, Lord CJ, Buerger H, Ashworth A, Reis-Filho JS: Tiling path genomic profiling of grade 3 invasive ductal breast cancers. Clin Cancer Res 2009, 15:2711-2722.

17. Shinnomiya T, Mori T, Arimaya Y, Sakabe T, Fukuda Y, Murakami Y, Nakamura Y, Inazawa J: Comparative genomic hybridization of squamous cell carcinoma of the esophagus: the possible involvement of the DlPI gene in the 13q34 amplicon. Genes Chromosomes Cancer 1999, 24:337-344.

18. Dohmae M, Reinsccke M, Michaea A, Allolio B, Solinas-Toldo S, Lichter P: Adrenocortical carcinoma is characterized by a high fre-
quency of chromosomal gains and high-level amplifications. Genes Chromosomes Cancer 2000, 28:145-152.

19. Michiels EM, Weiss MM, Hoovers JM, Baak JP, Voute PA, Baas F, Hermoen MA: Genetic alterations in childhood medulloblastoma analyzed by comparative genomic hybridization. J Pediatr Hematol Onccol 2002, 24:205-210.

20. Yoshida Y, Zhao C, Imoto I, Ueda M, Nagai H, Emi M, Inazawa J: TFDP1, CUL4A, and CDC16 identified as targets for amplification at 13q34 in hepatocellular carcinomas. Hepatology 2002, 35:1476-1484.

21. Melchor L, Alvarez S, Honrado E, Palacios J, Barroso A, Diez O, Osorio A, Benitez J: The accumulation of specific amplifications characterizes two different genomic pathways of evolution of familial breast tumors. Clin Cancer Res 2005, 11:8577-8584.

22. Abba MC, Fabris VT, Hu Y, Kittrell FS, Cai WW, Donehower LA, Melchor L, Alvarez S, Honrado E, Palacios J, Barroso A, Diez O: Gene expression data. Diaz-Uriarte R, Al-Shahrour F, Herrero J, Dopazo J: Breast cancer differentiate BRCA2-associated tumors. BMC Genomics 2007, 6:36.

23. Chen LC, Manjeshwar S, Lu Y, Moore D, Ljung BM, Kuo WL, Dair-keeh SH, Wemick M, Collins C, Smith HS: The human homologue for the Caenorhabditis elegans cul-1 gene is amplified and overexpressed in primary breast cancers. Cancer Res 1998, 58:3677-3683.

24. Mc Cormack O, Chung WY, Fitzpatrick P, Cooke F, Flynn B, Harrison M, Fox E, Gallagher E, Goldrick AM, Dervan PA, McCann A, Khan M: Cul-4A gene amplified and overexpressed in mouse and human breast cancer at a syn-tenic cluster mapping to mouse ch8A1 and human 13q34. Cancer Res 2007, 67:4104-4112.

25. Schindl M, Gnath M, Schoppmann SF, Horvat R, Bimer P: Over-expression of the human homologue for Caenorhabditis elegans cul-4 gene is associated with poor outcome in node-negative breast cancer. Anticancer Res 2007, 27:949-952.

26. Yaou K, Okamoto H, Arii S, Inazawa J: Association of over-expressed TFDSP1 with progression of hepatocellular carci- nomas. J Hum Genet 2003, 48:609-613.

27. Diez O, Osorio A, Duran M, Martinez-Ferrandis JJ, de la Hoya M, Santaraz R, Vegas D, Campos B, Rodriguez Lopez R, Velasco E, Cavatas A, Alonso C, San Roman JM, Gonzalez-Sarmiento R, Miner vante A, Alonso C, San Roman JM, Gonzalez-Sarmiento R, Mine- ra C, Stoppani Lyonnnet D, Pierga JY, Salmon R, Sastre-Garau X, Fourquet A, Delattre O, de Cremoux P, Auriol A: Identification of typical medullary breast carcinoma as a genomic sub-group of basal-like carcinomas, a heterogeneous new molecular entity. Breast Cancer Res 2007, 9:R24.

28. Kiperos ET, Landre LR, Wing JP, We HH, Hedgecock EM: cul-1 is required for cell cycle exit in C. elegans and identifies a novel gene family. Cell 1996, 85:829-839.

29. Gupta A, Yang LX, Chen L: Study of the G2/M cell cycle check-point in cancer. Anticancer Res 2007, 27:949-952.

30. Roche Applied Science [http://www.roche-applied-sci-ence.com]

31. Al-Shahrour F, Herrero J, Dopazo J: Over-expression of the human homologue for Caenorhabditis elegans cul-1 gene is amplified and overexpressed in primary breast cancers. Cancer Res 2007, 67:4104-4112.

32. Chen LC, Manjeshwar S, Lu Y, Moore D, Ljung BM, Kuo WL, Dairkee SH, Wemick M, Collins C, Smith HS: The human homologue for the Caenorhabditis elegans cul-1 gene is amplified and overexpressed in mouse and human breast cancer at a syntenic cluster mapping to mouse ch8A1 and human 13q34. Cancer Res 2007, 67:4104-4112.

33. Melchor L, Alvarez S, Honrado E, Palacios J, Barroso A, Diez O, Osorio A, Benitez J: The accumulation of specific amplifications characterizes two different genomic pathways of evolution of familial breast tumors. Clin Cancer Res 2005, 11:8577-8584.

34. Hellemsans M, Mortier G, De Paepe A, Speleman F, Vandonosopele J: qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. Genome Biol 2007, 8:R19.

35. Fridlyand J, Snijders MJ, Ylstra B, Li H, Oshken A, Segreaves R, Daigee K, Tokuyasu T, Ljung BM, Jain AN, McLennan J, Ziegler J, Chin K, Devinsies S, Feller H, Gray JW, Waldman F, Pinkel D, Albert- son DG: Breast tumor copy number aberration phenotypes and genomic instability. BMC Cancer 2006, 6:36.

36. Vincent-Salomon A, Gruel N, Lucchesi C, MacGrogan G, Dendale R, Sigal-Zafrani B, Longi M, Raynel V, Pierreon G, de Mascarel I, Taris C, Stoppa-Lyonnet D, Pierga JY, Salmon R, Sastre-Garau X, Fourquet A, Delattre O, de Cremoux P, Auriol A: Identification of typical medullary breast carcinoma as a genomic sub-group of basal-like carcinomas, a heterogeneous new molecular entity. Breast Cancer Res 2007, 9:R24.