Chapter 6

Genomic signature of BRCA1 deficiency in sporadic basal-like breast tumors.

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About 10–20% of all breast carcinomas show a basal-like phenotype, while ~90% of breast tumors from BRCA1-mutation carriers are of this subtype. There is growing evidence that BRCA1-mutated tumors are not just a specific subset of the basal-like tumors, but that (the majority of) basal-like tumors show a dysfunctional BRCA1 pathway. This has major treatment implications, because emerging regimens specifically targeting DNA repair mechanisms would then be most effective against these tumors. To further understand the involvement of BRCA1 deficiency in sporadic basal-like tumors, we investigated 41 basal-like tumors for BRCA1 mRNA expression by quantitative real-time polymerase chain reaction, BRCA1 promoter methylation, their genomic profile by array-CGH, and gene expression levels by whole genome expression arrays. Array-CGH results were compared to those of 34 proven BRCA1-mutated tumors. Basal-like tumors were subdivided into two equal groups: deficient and proficient in BRCA1 gene expression. The chromosomal makeup of BRCA1 deficient sporadic basal-like tumors was similar to that of BRCA1-mutated tumors. BRCA1 proficient sporadic basal-like tumors were more similar to nonbasal-like tumors. Only half of the basal-like breast tumors are actually deficient in BRCA1 expression. Gain of chromosome arm 3q is a marker for BRCA1 deficiency in hereditary and sporadic breast tumors.

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Introduction

Breast cancer is the most frequently occurring cancer among women in the western world. It is a heterogeneous disease, consisting of several tumor subtypes. Identification and characterization of these subtypes are important to understand the pathogenesis of the disease and obtain better treatment options in the future. One of the breast cancer subtypes is called "basal-like" and describes an aggressive tumor group with poor prognosis. Basal-like breast tumors are characterized by the expression of markers often found in normal basal/myoepithelial cells (1) and the absence of the expression of estrogen receptor (ER), progesterone receptor (PR), and ERBB2 (HER2/neu) (2, 3). Because of the lack of expression of these receptors, this group is often referred to as "triple-negative". Among the sporadic breast cancers, the basal-like phenotype represents ~15% of the invasive tumors (3, 4). However, among the hereditary *BRCA1*-mutated breast tumors, this subtype accounts for 80–90% of cases (5, 6). Gene-expression profiles of *BRCA1*-mutated breast tumors show many similarities to those of sporadic basal-like tumors (7), and it has been shown that *BRCA1* mRNA expression is lower in most basal-like tumors compared to matched controls (8). This indicates that loss of function of *BRCA1* is important in basal-like tumors.

Sporadic basal-like tumors can loose *BRCA1* by various mechanisms, such as gene mutation

or promoter hypermethylation. Although in ~10% of the basal-like breast tumors a *BRCA1* mutation can be found (9), screening for gene mutations is generally not allowed in sporadic cancer cases where the patient did not give any informed consent. Furthermore, investigating promoter hypermethylation is not part of routine diagnostics for sporadic breast cancer, although it can be found in a substantial proportion of breast cancer patients (8, 10, 11). We therefore explored whether we could identify a general marker for *BRCA1* deficiency in this study.

Because *BRCA1* is involved in DNA repair by homologous recombination, loss of function will result in accumulation of DNA damage and chromosomal instability. As we and other researchers have shown, *BRCA1*-mutated tumors develop a distinct pattern of chromosomal aberrations (12-17). Some of these aberrations are similar to those of sporadic basal-like tumors (18, 19). However, the use of different detection techniques, control groups, and study designs in these studies makes direct and quantitative comparison between the sporadic basal-like and *BRCA1*-mutated tumors difficult, if not impossible. Thus far, the similarity between these two groups concerning copy number alterations remains elusive.

It would be of clinical and biological relevance to determine whether all or a fraction of the sporadic basal-like tumors are similar to the hereditary *BRCA1*-mutated tumors. The exact location of associated chromosomal aberrations and gene expression changes would result in a better understanding of tumorigenesis due to *BRCA1* deficiency in hereditary and sporadic basal-like tumors and may finally lead to the identification of common therapeutic targets. It has already been shown that breast cancer patients diagnosed with a *BRCA1*-mutation are more sensitive to DNA damage-based chemotherapy than sporadic tumors (20). Additionally, evidence for poly(ADP-ribose) polymerase (PARP) inhibitors efficiently targeting *BRCA1* deficiency is emerging quickly (21). In this study, we show the correlation between copy number alterations of basal-like sporadic breast carcinomas and their *BRCA1* mRNA expression levels.

Materials and methods

Tumor specimens

This study includes two groups of breast cancer cases that were all negative for ER, PR, and ERBB2 expression by immunohistochemistry (IHC) and scored as histological grade III. The first group consists of 41 sporadic basal-like breast tumors of invasive ductal carcinoma (IDC) type, defined as being sporadic as having no family history for any type of cancer, with a mean age at diagnosis of 48 years (range, 26–82), from which gene expression and histopathological data were available from an earlier study from our institute (2). The second group includes 34 breast carcinomas (IDC) from patients with a confirmed pathogenic *BRCA1* germ-line mutation and with a mean age at diagnosis of 38 years (range, 27–61). mRNA, and therefore gene expression data, was not available.

As an additional control, *BRCA1* gene-expression levels were measured in 83 unselected luminal sporadic tumors (IDC) by qRT–PCR, taken from an unrelated study from our institute (22), and included individuals with a mean age at diagnosis of 46 years (range, 27–78). Molecular breast cancer subtypes were determined by the subtype single sample predictor developed by Hu *et al.* (23) for both the basal-like and luminal tumor groups.

Table 1 – Median number of aberrations per tumor group.

Tumor group	Average	Range	StDev	t-test	p-value
Number of aberrations					
Basal-like (n=41)	82.3	48-129	15.1	B1 vs BL	3.0E-03
Basal-likeB1-low (n=22)	90.0	72-129	12.1	B1 vs $BL^{b1\text{-}low}$	0.39
Basal-likeB1-high (n=19)	73.4	48-95	13.4	B1 vs $BL^{b1\text{-}high}$	9.4E-07
BRCA1-mutated (n=34)	90.9	69-113	10.1	B1 vs C	2.8E-07
Non-basal-like (n=23)	75.0	58-103	10.2	$BL^{b1\text{-}low}$ vs $BL^{b1\text{-}high}$	7.5E-08
				$BL^{b1\text{-}low}$ vs C	3.3E-05
				$BL^{b1\text{-}high}$ vs C	0.33
Number of losses					
Basal-likeB1-low (n=22)	43.9	33-69	7.4	$BL^{b1\text{-}low}$ vs $BL^{b1\text{-}high}$	4.6E-4
Basal-likeB1-high (n=19)	33.1	15-52	10.1		
Number of gains					
Basal-likeB1-low (n=22)	28.5	19-39	5.9	$BL^{b1\text{-}low}$ vs $BL^{b1\text{-}high}$	0.86
Basal-likeB1-high (n=19)	28.9	19-45	7.4		
Number of amplifications					
Basal-likeB1-low (n=22)	17.8	7-34	7.6	$BL^{b1\text{-}low}$ vs $BL^{b1\text{-}high}$	0.47
Basal-likeB1-high (n=19)	15.8	2-37	9.3		

P-values are calculated between tumor groups using 2-tailed t-tests. Number of aberrations in basal-like$^{B1\text{-}low}$ and basal-like$^{B1\text{-}high}$ tumors were also separately analyzed for losses, gains, and amplifications. B1=BRCA1-mutated, BL=Basal-like, C=non-basal-like, StDev=Standard Deviation.

As a control group for chromosomal aberrations, array-CGH profiles from 23 sporadic, histological grade III, and carcinomas (IDC) were used. These tumors expressed either one or a combination of ER, PR, and ERBB2 (Supporting Information Table 1) and with a mean age at diagnosis of 45 years (range, 32–60). This group is further referred to as the nonbasal-like tumors.

All experiments involving human tissues were conducted with the permission of the institute's medical ethical advisory board.

Pathological review

The presence of ER, PR, and ERBB2 were determined by revision of immunohistochemical staining that was previously performed using a standard clinical procedure with antibodies: ER AB-14 clone 1D5 + 6F11, titer 1:50 (Neomarkers); PR clone PR-1, titer 1:400 (Immmunologic); and c-erbB-2 clone SP3, titer 1:25 (Neomarkers), respectively. For simplicity, IHC scoring was divided into two classes. If ≥1% of the tumor cells expressed ER or PR, the tumor was scored as positive (+); otherwise, the tumor was scored as negative (-) for the corresponding staining, according to Viale *et al.* (24). ERBB2 scoring was performed according to ASCO/CAP and oncoline guidelines (25, 26). A tumor was scored positive for ERBB2 when a 3+ staining was observed. When a 2+ staining was observed, CISH was performed to determine amplification (+ in case of six spots or more per nucleus) or no amplification (-). A 1+ or negative IHC staining was scored as negative (-). Tumor grade was determined using the modified Bloom–Richardson–Elston staging system (27).

DNA isolation and array-CGH

All sample material used for array-CGH experiments was formalin-fixed, paraffin-embedded tissue from the hospital's pathological archive, collected between 1985 and 2001. DNA was extracted by proteinase-K digestion after deparaffinization, and quality was tested using a multiplex PCR as previously described (13, 28). Tumor and reference DNA were labeled with Cy5 and Cy3, respectively, co-hybridized to a microarray containing 3.5k BAC/PAC-derived DNA segments covering the whole genome with an average spacing of 1 Mb and processed as already described (29). Microarray data were deposited in NCBIs Gene Expression Omnibus and are accessible through GEO Series accession number GSE22401 (basal-like), GSE9021 (*BRCA1*-mutated), and GSE9114 (nonbasal-like).

Aberration detection and quantification

To analyze and visualize chromosomal aberrations, we determined breakpoint locations and estimated copy number levels using the CGH-segmentation algorithm by Picard *et al.* (30). These data are referred to as the "segmentation data". To call the copy number level of aberrations, profile-dependent cutoffs were used that were based on the SD of the middle 50% quantile of the segmented data as described by Chin *et al.* (31). The association of the frequency of a clone being at a neutral, lost, gained, or highly gained copy number between different tumor groups was calculated by using a 2 x 4 Fisher's exact (FE) test (32). Because adjacent BAC clones are expected to be highly correlated, a genomic region was called significant when at least five adjacent BAC clones were calculated to be significant with $p < 0.01$. Using this approach, identifying a region of 5 Mb by chance is < 0.01 (Benjamini), and copy number variations smaller than 5 Mb were also excluded from the analyses.

Methylation MLPA

Methylation of the *BRCA1* promoter was investigated using a methylation-specific MLPA kit according to the manufacturer's protocol (ME001B, MRC-Holland, The Netherlands). This kit includes probes against the gene promoters of *APC*, *ATM*, *BRCA1*, *BRCA2*, *CASP8*, *CD44*, *CDH13*, *CDKN1B*, *CDKN2A*, *CDKN2B*, *CHFR*, *DAPK1*, *ESR1*, *FHIT*, *GSTP1*, *HIC1*, *IGSF4*, *MLH1*, *PTEN*, *RARB*, *RASSF1*, *TIMP3*, *TP73*, and *VHL* and includes 15 reference probes. Basal-like tumors that show *BRCA1* promoter methylation were classified as BRCA1-deficient. The *BRCA1* mRNA expression levels of these samples were used to calculate the 95% reference range. Next, the reference range was applied to *BRCA1* mRNA expression levels of basal-like samples without *BRCA1* promoter methylation. Samples with expression levels inside the reference range were included into the *BRCA1*-deficient group, and samples outside the 95% reference range were classified as *BRCA1*-proficient.

Quantitative RT–PCR

Expression levels of *BRCA1* were assessed by quantitative real-time polymerase chain reaction (qRT-PCR) as a method independent of the microarray data to prevent array-based bias in the 41 basal-like and 83 luminal breast tumors. The TaqMan Gene Expression Assay for *BRCA1* (#Hs01556193_m1, Applied Biosystems, Foster City, CA) was used for this purpose. The reactions were performed according to the manufacturer's protocol with 10 ng cDNA (2 ng/µl) for each sample, resulting in an amplification

product of 59 nucleotides. Expression levels of β-actin and GAPDH were measured as endogenous controls, and cDNA from MCF-7 cells in different dilutions was used to obtain a standard curve. qRT-PCR runs were performed on the 7500 Fast System, and analyses were conducted using 7500 Fast Real-Time PCR Software version 1.3.1. Expression levels were calculated by the relative standard curve method.

Results

BRCA1 expression in basal-like tumors

To investigate whether basal-like tumors could be subdivided into groups based on their level of *BRCA1* expression, *BRCA1*-deficient tumors were identified by measuring *BRCA1* promoter methylation. Fourteen of the basal-like tumors (34%) showed *BRCA1* promoter methylation. Promoter methylation usually results in silencing of the gene. Indeed, all cases with methylated *BRCA1* promoter showed low-*BRCA1* mRNA expression compared to the remaining samples ($p < 5.0e-5$, two-sided t-test). We defined the basal-like tumors with methylated *BRCA1* promoter as the basal-like "BRCA1-low" group, which is subsequently referred to as the basal-like$^{B1\text{-}low}$ group. Using 95% reference range on their *BRCA1* expression levels, we determined which of the basal-like samples without *BRCA1* promoter methylation could be included in the basal-like$^{B1\text{-}low}$ group. All samples outside the 95% reference range were classified as basal-like "BRCA1-high" and are subsequently referred to as the basal-like$^{B1\text{-}high}$ group. As can be seen in Supporting Information Fig. 2, a binominal distribution could be used to describe the methylation results (methylated and unmethylated), because no continuous correlation was observed between levels of methylation and mRNA expression. Twenty-one samples (51%) were included in the basal-like$^{B1\text{-}low}$ group, and 20 samples (49%) were included in the basal-like$^{B1\text{-}high}$ group (Fig. 1).

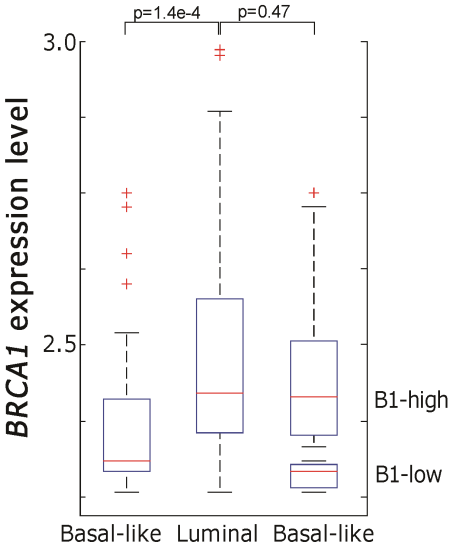

Figure 1 – BRCA1 mRNA expression. *Box plots showing relative BRCA1 mRNA expression in sporadic basal-like breast tumors (left, n=41), luminal breast tumors (middle, n=83), and the same basal-like breast tumor cohort separated on the basis of BRCA1 deficiency as described in Methods.*

Expression of *BRCA1* mRNA in the complete basal-like tumor cohort was significantly lower compared to the 83 sporadic luminal tumors ($p = 1.4e-4$, two-sided t-test) (Fig. 1). Median relative expression of *BRCA1* was 0.24 and 0.69 in basal-like and luminal breast tumors, respectively. For the basal-like$^{B1\text{-}low}$ group, the median expression level was 0.17, while it was 0.66 for the basal-like$^{B1\text{-}high}$ group, which is comparable to that of the luminal tumors ($p = 0.47$, two-sided t-test) (Fig. 1 and Support-

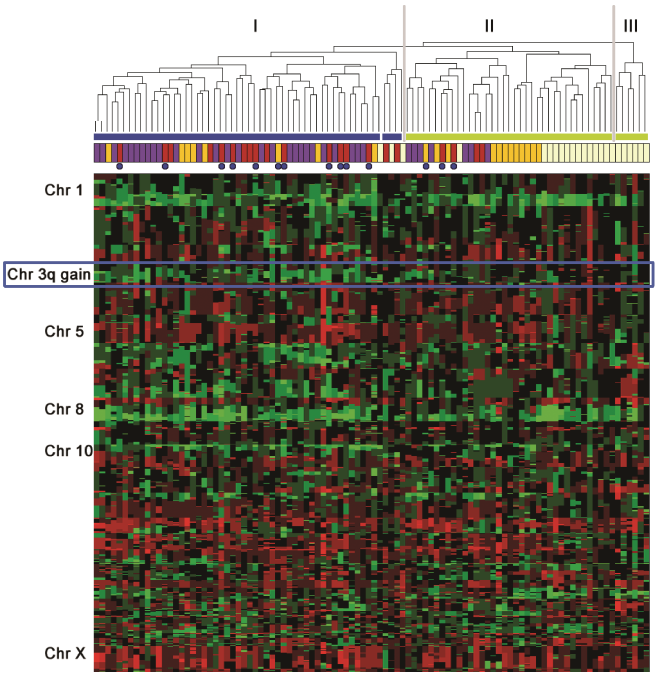

Figure 2 – Clustering of array-CGH profiles. *Unsupervised hierarchical clustering (complete linkage) of BRCA1-mutated ■, basal-like$^{B1\text{-}low}$ ■, basal-like$^{B1\text{-}high}$ ■, and nonbasal-like ■ tumors. While the BRCA1-deficient cases cluster together in cluster I ■, the BRCA1-proficient cases are located in clusters II-III ■. Shown is the heat map of the CGH segmentation data, where green is positive log2(ratio) and red negative. Here, gain on chromosome 3q has been highlighted, which has been found in BRCA1-deficient, and not BRCA1-proficient tumors, as a significant difference in later analyses. Blue circles indicate samples with methylation of the BRCA1 promoter ●.*

ing Information Table 1). These results indicate that approximately half of the basal-like breast tumors express BRCA1 at levels similar to luminal tumors and half express BRCA1 at a significantly lower level, if at all.

RASSF1 gene promoter methylation and *BRCA1* expression

Although promoter methylation of *BRCA1* was abundant in sporadic basal-like tumors, we could not detect it in nonbasal-like tumors (n = 23). Additionally, in a larger series (n > 150), we have seen that methylation of the *BRCA1* promoter in sporadic nonbasal-like tumors was rare (3%, unpublished data). Besides *BRCA1*, 23 other tumor suppressor genes were simultaneously investigated for promoter methylation including *RASSF1*. Methylation of the *RASSF1* promoter is reported to be less abundant in *BRCA1*-associated breast cancer (33). As shown in Supporting Information Table 1, a trend can be observed in our data set, such that the *RASSF1* promoter was more often methylated in basal-like$^{B1\text{-}high}$ tumors compared to basal-like$^{B1\text{-}low}$ tumors ($p = 0.034$, two-sided t-test, uncorrected for multiple testing). These data were similar to the methylation patterns of *BRCA1*-mutated tumors, in which methylation of the promoter of *RASSF1* was also absent; nonbasal-like tumors on the other hand often showed methylation of the *RASSF1* promoter (73%, unpublished data, n > 150). These results indicated that *BRCA1* expression could be correlated with methylation of the *RASSF1* promoter. Methylation states of all individual basal-like tumor samples are listed in Supporting Information Table 1.

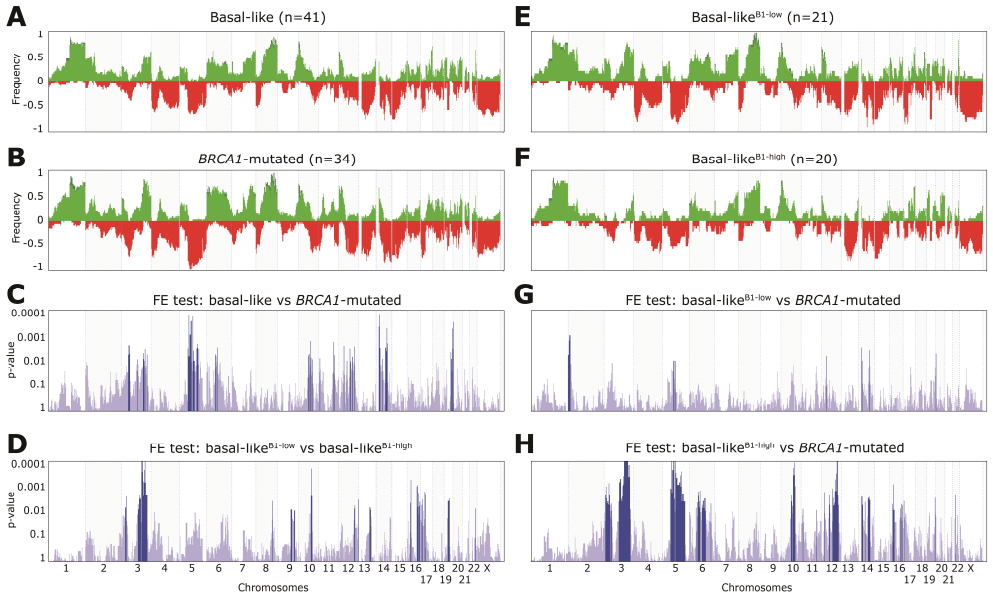

Figure 3 – Aberration frequencies. *Frequency plots of basal-like (A), BRCA1-mutated (B), basal-like$^{B1\text{-}low}$ (E) and basal-like$^{B1\text{-}high}$ (F) tumors, showing the amount of gain (green) and loss (red) along the whole genome. Fisher's exact test was used to determine the most significant regions between BRCA1-mutated and basal-like tumors (C), basal-like$^{B1\text{-}low}$ and basal-like$^{B1\text{-}high}$ tumors (D), BRCA1-mutated and basal-like$^{B1\text{-}low}$ tumors (G), and BRCA1-mutated and basal-like$^{B1\text{-}high}$ tumors (H). P-values are minus log10 transformed and depicted as light blue (p>0.01) or dark blue (p<0.01).*

Level of chromosomal imbalance is associated with level of BRCA1 expression

Histological high-grade tumors show extensive chromosomal imbalance (34). To determine whether there was a difference in the level of chromosomal imbalance among *BRCA1*-mutated, basal-like$^{B1\text{-}low}$, basal-like$^{B1\text{-}high}$, and grade III nonbasal-like control tumors, the number of aberrations (*i.e.*, calculated segments outside the profile-dependent thresholds) was counted (Table 1 and Supporting Information Fig. 3). Although all investigated tumors were histological grade III, the basal-like$^{B1\text{-}high}$ group showed significantly less aberrations compared to the *BRCA1*-mutated and basal-like$^{B1\text{-}low}$ groups ($p < 0.01$), but there was no significant difference when these tumors were compared to the nonbasal-like control tumors ($p = 0.33$). The average number of aberrations of the *BRCA1*-mutated and basal-like$^{B1\text{-}low}$ tumor groups was very similar ($p = 0.39$, Table 1). The difference in the number of aberrations was mainly caused by more losses found in basal-like$^{B1\text{-}low}$ tumors (Table 1). These results imply that the level of chromosomal imbalance is not dependent on histological grade, but on *BRCA1* status.

Copy number alterations in hereditary and sporadic breast tumors

To investigate the correlation among the genetic signatures (aberrations) of the different

tumor groups, unsupervised hierarchical clustering (complete linkage correlation) was performed on the whole-genome segmentation data of sporadic basal-like, *BRCA1*-mutated, and control tumors. Figure 2 shows that basal-like$^{B1\text{-}low}$ tumors cluster with *BRCA1*-mutated tumors, while many basal-like$^{B1\text{-}high}$ tumors cluster separately from the *BRCA1*-deficient tumors along with grade III nonbasal-like tumors. This indicates that similar aberrations are present in *BRCA1*-deficient tumors that are independent of the cause of the deficiency (*i.e.*, mutation or methylation). Additionally, tumors proficient in *BRCA1* develop a different signature of aberrations.

Next, supervised analyses were performed based on the frequency of copy-number alterations. We published previously that *BRCA1*-mutated tumors show a different spectrum of aberrations compared to the general population of sporadic breast cancer (13). In the present study, we also show that the spectrum of aberrations was very different when only compared to grade III nonbasal-like sporadic tumors (Supporting Information Fig. 1). Interestingly, the sporadic basal-like tumors were much more similar to the *BRCA1*-mutated breast tumors (Figs. 3A–3C). Panels A and B of Figure 3 display the genome-wide frequency of gain (green) and loss (red) in basal-like and *BRCA1*-mutated breast tumors, respectively. Most tumors in both groups showed the common breast cancer aberrations, namely, gain of chromosome arms 1q and 8q and loss of 8p. Moreover, previously identified aberrations specific for *BRCA1*-associated, ER negative, or basal-like tumors were also found, as represented by gains of regions in chromosome arms 3q, 6p, 10p, 12p, and 21q and losses of regions in 3p and 5q (see Supporting Information 2 for a detailed whole-genome description and exact locations). Figure 3C shows p-values calculated by Fisher's exact test based on the number of different aberrations in both groups. Several genomic regions (*e.g.*, located on 3q, 5q, 14q and 19q, see Supporting Information Table 2 for full list) were identified with significantly different frequencies between *BRCA1*-mutated and basal-like tumors ($p < 0.01$, indicated in dark blue). To determine whether these aberrations were correlated with *BRCA1* expression, the following two comparisons were performed.

First, an aberration frequency comparison was made between basal-like$^{B1\text{-}low}$ and basal-like$^{B1\text{-}high}$ tumors, which revealed several significantly different genomic regions (Fig. 3D and Supporting Information Table 2). In basal-like$^{B1\text{-}low}$ tumors, 3p24-p22.3, 3q13-q26.2, 13q22, 16p12-p11, and 16q22-q24 were more often gained, and 9q, 9q31.3-q33.1, 10q23.1-q23.31, and 12q23.3 were more often lost, compared to basal-like$^{B1\text{-}high}$ tumors.

Second, the basal-like tumor subgroups were compared to *BRCA1*-mutated tumors using a similar frequency analysis as outlined earlier. Figures 3E and 3F depict the aberration frequencies in the basal-like subgroups, whereas Figures 3G and 3H show the corresponding p-values calculated by Fisher's exact test (see also Supporting Information Table 2). Basal-like$^{B1\text{-}low}$ breast tumors were most similar to *BRCA1*-mutated tumors, and only two small genomic regions at 2p24-25 and 14q24 presented with a significantly different frequency ($P < 0.01$ at ≥ 5 adjacent BAC clones). Basal-like$^{B1\text{-}high}$ tumors showed many more aberrations with significantly different frequencies (Fig. 3H and Supporting Information Table 2), which included 3p21-p26, 3q11-26, 5q11-q33, 6q12-21, 10q21-q23, 12q13.13-q14.1, 12q21.2-q24.22, 14q11-q12, 14q23-q24, 16p12-p11, 16q22-q24, and 17p.

These results indicate that basal-like tumors

are quite similar to *BRCA1*-mutated tumors. However, differences among these groups are still present, which were only identified in basal-like$^{B1\text{-}high}$ tumors. Basal-like$^{B1\text{-}low}$ tumors are almost identical to *BRCA1*-mutated tumors.

H2B gene regulation is associated with *BRCA1* expression

Gene-expression data from Kreike and colleagues (2) were investigated to determine whether *BRCA1* mRNA expression relates to different gene-expression patterns in basal-like tumors. Differentially expressed genes in basal-like$^{B1\text{-}low}$ tumors could reveal biological processes associated with *BRCA1* deficiency. Additionally, the analysis of gene-expression patterns in basal-like$^{B1\text{-}high}$ tumors could elucidate differences within basal-like breast cancer.

To evaluate the statistical significance of gene-expression patterns between basal-like$^{B1\text{-}low}$ and basal-like$^{B1\text{-}high}$ tumors, the significance analysis of microarrays method (35) was used for all the 5830 significant genes. For a false discovery rate of 5%, delta was 0.488. Fifty-seven unique genes were found to be significantly downregulated in basal-like$^{B1\text{-}low}$ tumors when compared with basal-like$^{B1\text{-}high}$ tumors (Supporting Information 3) and none was upregulated. DAVID (36) was used to perform functional annotation clustering. From the significant genes, 7 (12.5%) were selected to be at the most significant cluster, Histone *H2B* ($p = 5.5e\text{-}9$, Benjamini), and consisted of the genes *H2BFS*, *HIST1H2BB*, *HIST1H2BD*, *HIST1H2BJ*, *HIST1H2BM*, *HIST1H2BO*, and *HIST1H2BE*.

Discussion

Breast carcinomas that are negative for ER, PR, and ERBB2, and of a basal-like subtype, are a distinct breast cancer subgroup associated with poor prognosis. Literature concerning the relationship between *BRCA1*-pathway deficiency and basal-like breast cancer has been increasing rapidly in the last few years (37-40). Because of their *BRCA1* deficiency, it is not surprising that basal-like and *BRCA1*-mutated breast tumors are similar in many aspects (20). It is of high clinical and biological interest to identify the similarities between these groups, which could lead to the identification of common therapeutic targets.

BRCA1 expression in basal-like tumors

Turner *et al.* (8) showed that basal-like breast tumors express less *BRCA1* mRNA compared to controls. Although slightly different definitions for basal-like and control cases were used, we can confirm these results and see a similar picture when comparing basal-like with luminal breast tumors (Fig. 1).

In our study, many basal-like breast tumor samples showed methylation of the *BRCA1* promoter (34%), which was significantly correlated with *BRCA1* gene downregulation. Although our methylation results accord with the findings of other studies, which showed that *BRCA1* promoter methylation is found in 32% of basal-like samples (10, 41), Turner *et al.* (8) detected a lower rate of 12% of ductal basal-like carcinomas exhibiting *BRCA1* promoter methylation. This difference might also be the result of the use of slightly different definitions for basal-like tumors. Besides promoter methylation, gene mutation can be the cause of loss of function and lowered gene expression. A recent study has shown that *BRCA1* is mutated in about 10% of sporadic basal-like breast tumors. It is therefore suggested that young women with early-onset triple-negative breast cancer are candidates for

mutation screening, regardless of family history of breast or ovarian cancer (9). Unfortunately, a limitation in our study was that permission for mutation screening was not granted for the sporadic tumors, because most samples where archived 10–20 years ago, and no indication of a hereditary mutation was present. From the remaining (non-methylated) samples, an additional 17% of cases also showed a down-regulated *BRCA1* expression, which may actually be due to a real *BRCA1* mutation. Separating basal-like$^{B1\text{-}low}$ cases based on methylation status, however, did not change any of our results (data not shown).

Forty-nine percent of all basal-like samples showed a *BRCA1* expression comparable to that of luminal tumors (Fig. 1).

BRCA1 proficiency

Although basal-like tumors are similar to *BRCA1*-mutated breast tumors in regard to gene expression profiles (7), our study, involving unsupervised analyses of genomic aberrations, showed that some basal-like tumors cluster away from *BRCA1*-mutated tumors and cluster together with nonbasal-like tumors (Fig. 2). These tumors belong primarily to the *BRCA1* expressing group (basal-like$^{B1\text{-}high}$). Analyses of aberration frequencies in basal-like$^{B1\text{-}high}$ tumors show a different pattern compared to *BRCA1*-mutated tumors. A significant difference involved a gain of chromosome arm 3q, an aberration very abundant in *BRCA1*-mutated and basal-like$^{B1\text{-}low}$ breast tumors, but almost always absent in basal-like$^{B1\text{-}high}$ and nonbasal-like breast tumors. Significant differences among the tumor groups were found not only for the frequency of specific aberrations, but also for the total number of aberrations. It can be presumed that the number of chromosomal aberrations (*i.e.*, level of genomic imbalance) is associated with the deficiency in a specific DNA repair pathway within a defined tumor population. Basal-like$^{B1\text{-}high}$ and control nonbasal-like tumors showed significantly fewer aberrations compared to basal-like$^{B1\text{-}low}$ and *BRCA1*-mutated breast tumors, indicating a difference in the handling of DNA repair. Frequent *RASSF1* promoter methylation in basal-like$^{B1\text{-}high}$ cases makes this group additionally more similar to nonbasal-like breast tumors where methylation of *RASSF1* promoter is common. *RASSF1* promoter methylation in basal-like$^{B1\text{-}low}$ and *BRCA1*-mutated breast tumors is rare, as noted by other researchers (33). Taken together, our results indicate that basal-like$^{B1\text{-}high}$ tumors are very similar to nonbasal-ike grade III breast tumors and less similar to proven *BRCA1*-deficient breast tumors.

BRCA1 deficiency

Our results indicate that basal-like breast tumors with *BRCA1* deficiency (basal-like$^{B1\text{-}low}$ tumors) show many similarities to hereditary *BRCA1*-mutated breast tumors in regard to genomic aberrations using direct comparisons. Unsupervised clustering and supervised analyses showed that the well-known BRCA1-specific aberrations located along 3p (loss), 3q (gain), 5q (loss), and 12q (loss) are shared between the two groups. Furthermore, the total number of aberrations and rare methylation of the *RASSF1* promoter is similar. When mRNA expression profiles of basal-like$^{B1\text{-}low}$ and basal-like$^{B1\text{-}high}$ tumors were compared, only a few differences were found. The most prominent gene cluster that was downregulated in basal-like$^{B1\text{-}low}$ tumors was histone *H2B*. Downregulation of histone gene expression has been shown to occur in response to DNA double-strand breaks (42). We postulate that, due to lack of functional *BRCA1*, accumulation of doublestrand breaks is high in

basal-like$^{B1\text{-}low}$ tumors, which keeps histone *H2B* downregulated. Because basal-like$^{B1\text{-}high}$ tumors show fewer aberrations and higher *BRCA1* gene expression levels, histone *H2B* is normally regulated in these tumors. However, because p53 deficiency alleviates H2B downregulation (42) and p53 is frequently mutated in basal-like and *BRCA1*-mutated breast cancer (43, 44), the correlation is counter intuitive, and the biological relevance of this observation needs to be further investigated. Nevertheless, these results indicate that the loss of *BRCA1* causes only minor and indirect gene expression changes in basal-like tumors and might explain why basal-like tumors always cluster together in gene-expression studies while greater heterogeneity is found among basal-like tumors in CGH studies.

Chromosome arm 3q as a BRCA1 deficiency marker

Several studies have investigated chromosomal aberrations in both sporadic basal-like (18, 19) and *BRCA1*-mutated tumors (12-14, 16, 17, 45). These studies show gain along 3q and 10p and loss along 5q as the most common aberrations in both groups [see also (46) Supporting Information Table 4]. This has led to the presumption that these tumor groups are similar in terms of chromosomal aberrations (40). However, in-depth analysis of these studies also reveals many discrepancies. It should be noted that the different study designs limit interstudy comparisons and hamper localization of the exact chromosomal boundaries of the aberrations that are shared by the tumor groups. In our study, we showed that only half of the basal-like tumors are very similar to *BRCA1*-mutated tumors. The genomic instability and specific aberrations that develop in basal-like tumors are strongly associated with *BRCA1* mRNA expression. Our cluster analyses even suggest that the genomic signature as a consequence of (the lack of) *BRCA1* expression could be as prominent as the dominant ER signature, because the basal-like$^{B1\text{-}high}$ tumors (ER-negative) cluster together with ER-positive, grade III, sporadic tumors instead of residing with tumors having equal ER, PR, and ERBB2 status.

In previous studies, we and other researchers have identified gain of chromosome arm 3q to be an important marker of *BRCA1*-mutated tumors when compared with sporadic tumors (12, 13, 17). In the study presented here, we showed that gain of 3q is not only present in hereditary tumors but also present as most significant and in the highest frequency in sporadic basal-like *BRCA1*-deficient tumors compared to basal-like sporadic tumors expressing *BRCA1*. Our results indicate that gain of 3q (smallest common region of gain: 3q24) could serve as a potential marker of *BRCA1* deficiency.

Conclusion

We conclude that only half of the basal-like breast tumors are actually deficient in *BRCA1* expression. Lack of *BRCA1* leads to a large amount of aberrations and accumulation of DNA damage, but not to many direct differences in gene-expression profiles. Gain of chromosome arm 3q is a marker for *BRCA1* deficiency in hereditary and sporadic breast tumors. Future research should include prescreening of basal-like tumors for gain of 3q to initiate additional *BRCA1* diagnostics (*i.e.*, mutation and promoter methylation screening) and to prove the clinical relevance of the similarity between *BRCA1*-mutated and basal-like$^{B1\text{-}low}$ breast tumors.

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