The retinoblastoma gene undergoes rearrangements in 

**BRCA1**-deficient basal-like breast cancer

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Competing interests
Authors declare no competing interests.
Abstract
Breast tumors from BRCA1 germ line mutation carriers typically exhibit features of the basal-like molecular subtype. However, the specific genes recurrently mutated as a consequence of BRCA1 dysfunction have not been fully elucidated. In this study, we utilized gene expression profiling to molecularly subtype 577 breast tumors, including 72 breast tumors from BRCA1/2 mutation carriers. Focusing on the RB1 locus, we analyzed 33 BRCA1-mutated, 36 BRCA2-mutated and 48 non-BRCA1/2-mutated breast tumors using a custom-designed high-density oligomicroarray covering the RB1 gene. We found a strong association between the basal-like subtype and BRCA1-mutated breast tumors and the luminal B subtype and BRCA2-mutated breast tumors. RB1 was identified as a major target for genomic disruption in tumors arising in BRCA1 mutation carriers and in sporadic tumors with BRCA1 promoter-methylation, but rarely in other breast cancers. Homozygous deletions, intragenic breaks, or microdeletions were found in 33% of BRCA1-mutant tumors, 36% of BRCA1 promoter-methylated basal-like tumors, 13% of non-BRCA1 deficient basal-like tumors, and 3% of BRCA2-mutated tumors. In conclusion, RB1 was frequently inactivated by gross gene disruption in BRCA1-related hereditary breast cancer and BRCA1-methylated sporadic basal-like breast cancer, but rarely in BRCA2-hereditary breast cancer and non-BRCA1-deficient sporadic breast cancers. Together, our findings demonstrate the existence of genetic heterogeneity within the basal-like breast cancer subtype that is based upon BRCA1-status.
**Introduction**

Breast cancer is a vastly heterogeneous disease with respect to tumor biology and clinical course. Research over the past decades has identified numerous genetic alterations, but the driving events in breast tumor development are still not fully known. Global gene expression profiling has established molecular subtypes associated with characteristic pathological and clinical traits (1-3). Subsequent studies have demonstrated different DNA copy number aberrations and degrees of genomic instability in these subtypes (4-6). The basal-like subtype comprises 10-20% of all breast cancers and typically have negative estrogen receptor (ER), progesterone receptor (PR) and HER2 status, poor prognosis and frequent genomic alterations (7). Breast tumors arising in patients with an inherited $BRCA1$-mutation are typically basal-like subtype, and share most features of sporadic basal-like breast cancer, whereas tumors from $BRCA2$-mutation carriers are generally ER and PR positive and of luminal phenotype (4, 8, 9). Diverse patterns of DNA copy number aberrations have been described in $BRCA1$- and $BRCA2$-mutated tumors supporting their evolution via distinct pathways (10). More recent data indicate that $BRCA1$-mutated and other basal-like tumors have almost indistinguishable DNA copy number profiles (4).

Mutational inactivation of both $RB1$ alleles is the primary molecular alteration causing the pediatric cancer retinoblastoma, but $RB1$ mutations have been found in a wide range of malignancies. However the exact role of $RB1$ in breast cancer is unclear and recent studies have found reduced RB1 protein expression in mainly basal-like and luminal B tumors (11). Interestingly, targeted deletion of both $RB1$ and $p53$ in mouse mammary progenitor cells induced triple negative or basal-like tumors.
addicted to RB1 loss (12). These studies suggest that RB1 inactivation is critical for a subset of breast cancers that possess a high proliferative capacity, although the underlying mechanism of RB1 inactivation remains unclear.

To address these issues, we analysed a comprehensive set of hereditary and sporadic breast cancers using genome-wide gene expression and genomic profiling. PAM50 stratification classified 85% of BRCA1-mutated as basal-like and 56% of BRCA2-mutated as luminal B. To identify genes that are specifically targeted in BRCA1-mutated tumors we performed genome-wide DNA copy number analysis of an extended set of BRCA1-mutated tumors. Genomic identification of targets in cancer (GISTIC) analysis highlighted chromosome 13q14.2 including the RB1 gene as a common region of deletion. Additionally, the finding was supported by an RB1 specific homozygous deletion in a BRCA1-mutated case. Application of an RB1 specific high-density oligonucleotide microarray identified intragenic rearrangements in 33% of BRCA1-mutated tumors, 36% of BRCA1-methylated cases, 13% of basal-like tumors and none in non-basal-like tumors. By FISH analysis a physical disruption of the RB1 gene was observed which subsequently lead to absence of protein expression. Overall, our results reveal that targeted loss of RB1 is a recurrent event in basal-like breast cancers and specifically frequent in BRCA1-deficient breast cancer, which may have therapeutic implications.

Material and Methods

Patients and tumor material

Freshly frozen breast-tumor tissues (n = 577) were obtained from the Southern Sweden Breast Cancer Group tissue bank at the Department of Oncology, Skåne
University Hospital, Lund, The Helsinki University Central Hospital, Finland and Landspitali University Hospital, Iceland. In addition, DNA from 72 breast tumors with known \textit{BRCA1} or \textit{BRCA2} mutations was obtained from the same departments. Tumor and patient characteristics are summarized in Supplementary Table 1. The study was approved by the regional Ethical Committee in Lund (reg. no. LU240-01 and 2009/658), waiving the requirement for informed consent for the study, by the Icelandic Data Protection Committee and the National Bioethics Committee of Iceland, and by the Helsinki University Central Hospital Ethical Committee (207/E9/07). For Icelandic and Finnish patients, written informed consent was obtained according to the national guidelines.

All cell lines were obtained from American Type Culture Collection except for SUM-149, which was obtained from the originator, S. Ethier (13), and L56Br-C1, which was established and characterized in our laboratory (14). All cell lines were used within 6 months of culturing after receipt. Cell line identities are further confirmed by the consistency of our global copy number profiles to those in the published literature. HCC1937, HCC1428, BT549, and L56Br-C1 were maintained in RPMI supplemented with 10\% FBS, MDA-MB-231 in DMEM supplemented with 10\% FBS, and MDA-MB-436 were cultured in Mccoys 5A supplemented with 10\% FBS. SUM-149 were maintained in Ham’s F12 supplemented with 5\% FBS, 5 \textmu g/ml insulin, and 1 \textmu g/ml hydrocortisone, and MCF-10A were cultured in DMEM/F12 supplemented with 5\% horse serum, 20 ng/ml EGF, 0.5 mg/ml hydrocortisone, 100 ng/ml cholera toxin, and 10 \textmu g/ml Insulin. All cell lines were cultured with penicillin/streptomycin.
Gene expression analysis

Global gene expression analysis of 577 breast tumors was performed using oligonucleotide microarrays (Gene Expression Omnibus, GEO, platform GPL5345) produced at the SCIBLU Genomics Centre at Lund University, Sweden, as described (15). Normalization and data analysis was performed as previously described (4). PAM50 classification was performed as described (16), using gene expression centroids obtained from Parker et al. (3). Gene expression data for the complete cohort of 577 cases are available as GSE25307.

BAC array-based comparative genomic hybridization (aCGH) analysis

BAC aCGH data for 359 cases, all included in the 577-sample gene expression data set, were obtained from GEO (GSE22133), and analyzed as described (4). In addition, genomic profiles for 72 breast tumors comprising 38 BRCA1-mutated and 34 BRCA2-mutated cases were generated using the same BAC aCGH platform and analysis steps as for GSE22133, resulting in total 58 BRCA1-mutated and 66 BRCA2-mutated cases with BAC aCGH profiles when combined with samples in GSE22133. Genomic Identification of Significant Targets in Cancer (GISTIC) (17) analysis was performed individually for BRCA1- and BRCA2-mutated cases as previously described (4) using a q-value threshold of 0.25. RB1 gene copy number loss in sporadic and BRCA1/BRCA2-negative familial breast tumors were investigated using the 305 non BRCA1- and BRCA2-mutated cases remaining in GSE22133 (4).

High-resolution aCGH analysis

Custom-designed 60-mer oligonucleotide high-resolution aCGH (HD-aCGH) arrays for interrogation of gene specific alterations were designed using the Agilent eArray
ver. 5.3 software (Agilent Technologies, Santa Clara, CA) similar as described (18). HD-aCGH analysis was performed on 1) four BRCA1-mutant breast cancer cell lines (L56Br-C1, HCC1937, MDA-MB-436, and SUM-149), 2) the basal-like BT549 cell line, 3) the BRCA2-mutant HCC1428 cell line, 4) 33 BRCA1- and 36 BRCA2-mutated breast tumors, 5) 15 non-BRCA 1/2 mutated basal-like breast tumors, 6) 11 non-hereditary basal-like breast tumors with BRCA1 promoter-methylation, 7) six non-hereditary luminal A breast tumors, 8) six non-hereditary luminal B breast tumors, 9) five non-hereditary HER2-amplified breast tumors, and 10) five non-hereditary normal-like breast tumors. Microarrays were processed as described (18). Breakpoint analysis was performed using circular binary segmentation ($\alpha = 0.01$) (19). Agilent probes were mapped to the UCSC build 18. All tumors profiled by HD-aCGH were also profiled by BAC aCGH.

FISH and immunohistochemical analysis

FISH analysis was performed as described (20) on L56Br-C1 and SUM-149 cells using BAC probes RP11-90M2, RP11-120G8 and RP11-639F5 positioned immediately centromeric of RB1, and RP11-108P5, RP11-165D7, and RP11-90K7 positioned immediately telomeric of RB1. IHC analysis of RB1 protein expression on different TMAs was performed according to manufacturer’s instructions (4H1, Cell Signaling).

BRCA1 promoter-methylation analysis

BRCA1 promoter hypermethylation analysis was performed using a PSQ HS 96 pyrosequencing system (Biotage), and included two CpG island regions as described (13). For each tumor sample to be determined as BRCA1 methylated a methylation
allele frequency ≥10% was required. Additionally, a fully methylated as well as an unmethylated sample was included as controls. All electropherograms were manually checked.

**TP53 and RB1 sequencing**

Exon four to ten of the *TP53* gene and all coding exons of *RB1* were analyzed by Sanger sequencing using an ABI3130XL sequencer. Primers were designed using the Primer3 web software and sequences were evaluated using Sequencher software (Genes Codes Corp.).

**Western Blot analysis**

Whole-cell lysates were resolved by SDS-PAGE and transferred to PVDF. Primary antibodies directed against RB1 (4H1, Cell Signaling) and β-tubulin (Tu27, Covance) were used.

**Statistical Analysis**

Kaplan-Meier analysis and Fishers Exact Test were performed in R.

**Results**

*Comprehensive Gene Expression Profiling of Hereditary Breast Cancer*

Our analysis of global gene expression profiles from a cohort of 577 breast cancers (herein after referred to as the 577-set; Supplementary Table 1), including 34 tumors from *BRCA1* germline mutation carriers, confirmed the strong association between *BRCA1*-mutation and the basal-like subtype (\(P=2\times10^{-30}\), Fisher’s Exact Test) (Figure 1). Additionally, we found that *BRCA1* gene promoter-methylation was strongly
associated to basal-like classification (21 of 23 tumors; $P=4 \times 10^{-13}$, Fisher’s Exact Test) (Figures 1A and B). Moreover, BRCA1-mutation and promoter-methylation were mutually exclusive. Although BRCA2-mutated tumors were found in all subtypes, 56% of them were classified as luminal B ($P=7 \times 10^{-9}$, Fisher’s Exact Test) (Figure 1C). In contrast, tumors from BRCA1/BRCA2-negative familial cases were distributed equally across all molecular subtypes (Figure 1A).

**Molecular Heterogeneity in BRCA1-mutated and Basal-like Breast Cancer**

We were interested in examining the molecular heterogeneity of basal-like breast cancer, and in particular focusing on tumors with and without BRCA1-dysfunction. Within the gene expression data, we identified 321 genes (FDR=20%) differentially expressed between BRCA1-mutant or BRCA1-methylated basal-like tumors compared to basal-like tumors with wild type BRCA1. Interestingly, the expression of RB1, BRCA1, CDK6 and CCND1 were reduced in the BRCA1-deficient group. To further examine this heterogeneity, we used BAC-aCGH on 58 such tumors using BAC aCGH. GISTIC (17) analysis revealed 23 deleted regions of which many have previously shown to characterize BRCA1-mutated breast cancer (4), including 3p21.31, 5q11.2 and the RB1 region at 13q14.2 where the latter was highlighted by an RB1 gene specific homozygous deletion and high frequency of heterozygous copy number loss (66%) (Figure 2).

**Identification of Genetic Rearrangement of the RB1 Gene**

Based on our previous results (13) suggesting that tumors with DSB repair deficiency potentially have a higher frequency of intragenic rearrangements we designed a HD-aCGH assay with 1041 probes covering RB1 with an average spacing of 171bp for
identification of small intragenic $RB1$ lesions not detectable by BAC aCGH. Remarkably, HD-aCGH analysis of 33 $BRCA1$- and 36 $BRCA2$-mutated tumors revealed intragenic breakages and homozygous multi-exon deletions in $RB1$ in nine (27%) and two (6%) cases, respectively, of the 33 $BRCA1$-mutated tumors, all being of the basal-like subtype. There was one $BRCA2$-mutated case with RB1 intragenic break; interestingly, this BRCA2-case was both ER-negative and classified as basal-like by gene expression profile. Furthermore, sequencing all $RB1$ exons in the 22 $BRCA1$-mutated tumors without intragenic $RB1$ breaks identified one additional case with a 28bp frame-shift deletion in exon 4 of $RB1$. Taken together, 36% of $BRCA1$-mutated tumors were found to harbor an $RB1$ gene alteration, while only 3% of $BRCA2$-mutated tumors ($P=3.64\times10^{-4}$, Fisher´s Exact Test). To relate these findings to breast cancer without known $BRCA1$ or $BRCA2$ mutations, we used HD-aCGH to screen another 48 tumors representing all the five established molecular subtypes (Table 1). Consistently, $RB1$ intragenic breaks were observed only in basal-like tumors and predominantly in $BRCA1$ promoter-methylated basal-like tumors (36%) compared to $BRCA1$ non-methylated basal-like tumors (13%) (Table 1). Moreover, we screened the $TP53$ gene to investigate correlation between $RB1$ alterations and $TP53$ mutation. No difference in $TP53$ mutation frequency in $RB1$ wildtype or $RB1$ altered was found ($P=0.60$, Fisher´s Exact Test).

**Physical Disruption of $RB1$ Validated in BRCA1-deficient Cell Lines**

Intragenic $RB1$ breaks were also found in three $BRCA1$-mutated breast cancer cell lines (L56Br-C1, HCC1937, MDA-MB-436) and one basal-like cell line (BT549), whereas a fourth $BRCA1$-mutated (SUM-149) and a $BRCA2$-mutated (HCC1428) cell line showed no alterations by HD-aCGH analysis (Figure 3A). To corroborate the
RB1 gene breakage, we performed dual-color FISH analysis on metaphase spreads from L56Br-C1 and SUM-149 cells using six BAC probes mapping to either the 5’ or 3’ ends of the RB1 gene. A clear separation of fluorescent BAC probes was identified in L56Br-C1 cells indicating a physical disruption of the RB1 gene, whereas no such separation was observed in SUM-149 cells in line with the HD-aCGH results (Figure 3B). Furthermore, confirming that the intragenic breaks result in loss of functional protein, no RB1 protein was detectable by Western blot in the cell lines with intragenic breakage in RB1 (Figure 3C).

RB1 Protein Expression in Breast Cancer

The correlation between BRCA1-deficiency, intragenic RB1 alterations and RB1 protein loss was further substantiated by immunohistochemical (IHC) analysis of a tissue microarray (TMA) comprising 21 BRCA1-mutated tumors of which ten were also analyzed by HD-aCGH. Thirteen of 21 cases (62%) displayed absent or low RB1 protein expression (0-10% staining, Figure 4A), and of the ten HD-aCGH analyzed cases three harbored an intragenic RB1 rearrangement and one an RB1 gene mutation resulting in reduced RB1 protein expression in all four cases (<10% positive cells) (Figures 4B and C). To extend the analysis of RB1 inactivation beyond BRCA1-mutated tumors we first analyzed RB1 protein expression in a TMA comprising 84 samples for which we also had molecular subtyping data by gene expression profiling. These included 20 basal-like, 12 HER2-enriched, 14 luminal A, 13 luminal B, 14 normal-like and 11 unclassified cases. Strikingly, only basal-like and luminal B tumors displayed absent or low RB1 protein expression (<10% positive cells) with basal-like tumors showing the highest frequency (\(P=6.02 \times 10^{-5}\), Fisher’s Exact Test) (Figure 5A). One BRCA1 promoter-methylated case with RB1 gene breakage was
included in the 84-sample TMA; similar to BRCA1-mutated cases with abrogated RB1, this case had no RB1 protein expression (0% positive cells) (Figure 5B).

**Clinical Impact of RB1 Gene Copy Number Loss in Breast Cancer**

In line with RB1 protein expression levels, the highest frequency of RB1 gene copy number loss was observed in basal-like and luminal B tumors when investigated in a set of 305 sporadic and BRCA1/BRCA2-negative familial breast cancers (4). RB1 deletion was not associated with poor clinical outcome in the 305 tumors from Jönsson et al. (4) overall (P=0.47, log-rank test), however, in the subset of ER-positive tumors RB1 deletion was associated with a worse outcome (P=0.046, log-rank test) reflecting the high frequency of RB1 deletion in the clinically aggressive luminal B tumors.

**Discussion**

Early studies established the fact that breast tumors from BRCA1 and BRCA2 germline mutation carriers have distinct genomic and phenotypic characteristics (9, 21-23). Subsequently, it was shown that the majority of BRCA1-mutated tumors belong to the basal-like subtype (2, 4), although it is still unclear whether there are molecular patterns that distinguish them from non-BRCA1-mutated basal-like breast cancer. Here, we report findings to support the hypothesis that BRCA1-deficient breast cancer in fact develops through distinct genetic pathways.

In our large 577-set of PAM50-classified breast cancers we reinforce the strong correlation between BRCA1-mutations and the basal-like subtype and, additionally, demonstrate a strong association between BRCA1-methylation and the basal-like
subtype as well as between BRCA2-mutations and the luminal B subtype. Genomic instability and frequent low-level copy number changes of typical patterns are hallmarks of BRCA1 and BRCA2 tumors (10), however, the identity of specifically targeted genes has been illusive. We recently demonstrated that the PTEN tumor suppressor gene is disrupted by rearrangements in a fraction of BRCA1-deficient tumors (13), which encouraged us to search for additional similar hits. GISTIC analysis of genome-wide DNA copy number data from 58 BRCA1-mutated tumors revealed several affected regions, including 13q14.2, highlighted by a homozygous deletion at the RB1 locus. Although loss of pRB expression in basal-like breast cancers has been reported (24), the mechanisms of RB1 inactivation has not been clearly demonstrated. Thus, we designed a high-density oligomicroarray covering RB1 at a resolution of 171bp and, interestingly, identified RB1 rearrangements in one third of BRCA1-mutated cases. Dual-color FISH analysis of metaphase chromosomes from BRCA1-mutated cancer cells confirmed the physical disruption of RB1. These aberrations are expected to result in absence of pRB protein expression, a feature commonly observed in basal-like tumors, but also in luminal B tumors (24). We found only one of 36 BRCA2-mutated tumors harbored an intragenic RB1 breakage. Interestingly, this particular case was one of the few BRCA2-tumors classified as basal-like. Furthermore, by analysis of 48 non-BRCA1/2-mutated tumors, representing all molecular subtypes, RB1 breakages were only observed in basal-like cases. The fact that these preferentially were cases with BRCA1 methylation further substantiates the strong selection for dual BRCA1 and RB1 inactivation, and identifies RB1 disruption as a rather specific feature of BRCA1-dysfunctional breast cancer whether it is by germline mutation or somatic promoter hypermethylation. It is interesting to note that one of two tumors with RB1 breakage and unconfirmed BRCA1 inactivation
was classified as basal-like and obtained from a patient with family history of breast and ovarian cancer, suggesting that an unrevealed \textit{BRCA1} mutation may exist also for this deviating case. Accordingly, none of the luminal B classified tumor harbored \textit{RB1} breakage, despite frequent loss of pRB expression, suggesting that other genetic, epigenetic or post-translational mechanisms are operational in the luminal B subtype of breast cancer.

An intriguing observation was the similarly high frequency of \textit{RB1} breakages in \textit{BRCA1}-mutated and \textit{BRCA1}-methylated cases, which may reflect that \textit{BRCA1} methylation is an early event that drive or influence tumor progression in a manner similar to a germline \textit{BRCA1} mutation. The genetic association of \textit{BRCA1} and \textit{RB1} was further supported by the demonstration of a physical interaction between these two major tumor suppressors (25). Thus, the highly proliferative capacity of basal-like \textit{BRCA1}-mutated breast cancers could potentially be due to a combination of both \textit{BRCA1} and \textit{RB1} abrogation. It has also been hypothesized that combined inactivation of \textit{RB1}, \textit{TP53} and \textit{BRCA1} confer a basal-like phenotype. However, no difference in the frequency of somatic \textit{TP53} mutations was observed between \textit{BRCA1}-mutated tumors with and without \textit{RB1} gene breakage (46% mutated in both groups). Our findings are contradictory to results from a recent study of ovarian cancer (26), where \textit{BRCA1/2} inactivation was found to be mutually exclusive to \textit{RB1} inactivation (and \textit{CCNE1} activation), which may suggest the role of tumor-type specific oncogenic networks.

In summary, our study reveals that targeted loss of \textit{RB1} is a recurrent event in basal-like breast cancers, a subgroup characterized by high genomic instability. \textit{RB1}
intragenic breaks are only observed in basal-like tumors and predominantly in 
BRCA1-mutated or BRCA1 promoter-methylated cases, resulting in significantly 
reduced or absent pRB expression. These novel results define a genetic heterogeneity 
within the basal-like subtype, based on BRCA1-status. This may have implications for 
understanding differential response to chemotherapy of triple-negative or basal-like 
breast cancer, to drugs that target DNA repair deficiency or for development of new 
drugs that compensate for RB1 dysfunction.

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References


**Figure Legends**

**Figure 1.** BRCA1-deficient breast cancer is associated with the basal-like molecular subtype. A) Hierarchical clustering analysis of 577 breast tissue samples using the PAM50 gene set (3). In the heatmap rows correspond to PAM50 genes and columns to samples. Red indicates elevated expression and green reduced expression. Tumors are broadly divided into the five major molecular subtypes, basal-like, HER2-enriched, luminal A and B and normal-like as shown by corresponding PAM50 subtype classification. BRCA1-mutated and BRCA1-methylated cases are as indicated typically classified into the basal-like subtype, BRCA2-mutated cases are generally classified as luminal B, while sporadic and BRCA1/BRCA2-negative familial breast
tumors (BRCAx) are distributed equally across all molecular subtypes. B) Fisher’s Exact Test for investigating an association between BRCA1-deficiency including BRCA1-mutation or gene promoter-methylation and the basal-like subtype. C) Fisher’s Exact Test for investigating an association between BRCA2-mutation and the luminal B subtype.

Figure 2. BAC aCGH analysis of BRCA1-mutated breast cancer. A) Identification of significant regions of copy number loss in 58 BRCA1-mutated tumors analyzed by BAC aCGH using Genomic Identification of Significant Targets In Cancer (GISTIC) analysis. GISTIC identified 23 significant regions of copy number loss with a q-value <0.25, including the RB1 locus, based on the G-score pattern that integrates amplitude and frequency of copy number alterations. B) Homozygous deletion of RB1 in a BRCA1-mutated breast tumor identified using BAC array analysis.

Figure 3. Validation of RB1 intragenic rearrangements in breast cancer cell lines and its effect on RB1 protein expression. A) HD-aCGH RB1 gene specific copy number plots of L56Br-C1 (left) and SUM-149 cells (right). In L56Br-C1 cells a intragenic rearrangement in RB1 is observed, highlighted by colored HD-aCGH probes (red). B) Dual color split FISH indicate physical disruption of the RB1 locus in L56Br-C1 cells (arrows, lower left panel) while being intact in SUM-149 cells (lower right panel). Top 3 panels show corresponding FISH results from metaphase spreads of three normal samples. C) Western blot analysis confirm loss of RB1 protein in cells harboring RB1 intragenic break while cells without an intragenic break express RB1 protein.
Figure 4. RB1 gene breakage leads to lack of RB1 protein expression in BRCA1-mutated tumors. A) RB1 protein expression as determined by IHC analysis a 21-sample TMA comprising BRCA1-mutated cases (left). Cases are graded into four levels based on percentage of positively stained cells with numbers per group shown on the top x-axis. B) Examples of RB1 IHC data from BRCA1-mutated tumors. C) RB1 copy number profile for BRCA1-mutated tumor Ca4477 not showing any copy number alteration corresponding to the IHC data where the tumor have more than 90% of the cells expressing RB1. Ca7135 and Ca13714 harbor intragenic DNA alterations and correspondingly absent RB1 protein expression. A CBS segmentation line is superimposed in red in all three plots and red arrow indicates the break.

Figure 5. Frequency of RB1 gene copy loss and protein expression in non-hereditary breast cancer (including seven BRCA1 methylated cases) stratified by molecular subtype. A) Bar plot of RB1 protein expression for 84 sporadic breast tumors stratified by PAM50 molecular subtypes. Highest frequency of tumors displaying absent or low RB1 expression (<10% positive stained cells) was found in basal-like tumors. B) RB1 protein expression of Ca11822 being BRCA1 promoter methylation positive with <10% stained cells. C) HD-aCGH profile of RB1 in Ca11822 showing an intragenic DNA alteration (red). D) Frequency of RB1 gene copy number loss for 305 sporadic and BRCA1/BRCA2-negative familial cases in GSE22133 (4) stratified by molecular subtypes, showing frequent RB1 copy number loss in basal-like and luminal B tumors. P-value calculated by chi-square test.
Tables

Table 1. Frequency of RB1 gene rearrangement in breast cancer subgroups analyzed by HD-aCGH.

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<th>Subgroup</th>
<th>Number of cases</th>
<th>RB1 alteration, n (%)</th>
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<tr>
<td>BRCA1-mutated</td>
<td>33</td>
<td>12 (36)*</td>
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<tr>
<td>BRCA2-mutated</td>
<td>36</td>
<td>1 (3%)</td>
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<td>BRCA1-methylated basal-like</td>
<td>11</td>
<td>4 (36%)</td>
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<td>Basal-like</td>
<td>15</td>
<td>2 (13%)</td>
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<td>0</td>
</tr>
<tr>
<td>Luminal B</td>
<td>6</td>
<td>0</td>
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* One case harbors a 28bp deletion detected by Sanger sequencing.
FIGURE 1

A)

B)

C)

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<th>Basal-like</th>
<th>Non basal-like</th>
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<td>BRCA1-deficient</td>
<td>49</td>
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<td></td>
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FIGURE 4

A) % samples

B) >90% cells stained <10% cells stained <10% cells stained

C) Ca4477

Ca7135

Ca13714

RB1 exons

RB1 exons

RB1 exons
A) RB1 IHC staining, <10% positive cells

Baseline-like (n= 20)
Normal-like (n= 14)
Unclassified (n= 11)
HER2-enriched (n= 12)

B) Log(2) ratio

C) Exons

D) % samples with RB1 CN loss

Luminal A (n= 90)
Luminal B (n= 47)
Unclassified (n= 46)
HER2-enriched (n= 34)
The retinoblastoma gene undergoes rearrangements in BRCA1-deficient basal-like breast cancer

Goran Jonsson, Johan Staaf, Johan Vallon-Christersson, et al.

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