# BRCA1 interacts with FHL2 and enhances FHL2 transactivation function

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**Abstract** **Germ-line mutations in *BRCA1* are associated with an increased lifetime risk of developing breast and/or ovarian tumors. The BRCA1 gene product is a 220-kDa protein that contains a tandem of two BRCA1 C-terminal (BRCT) domains required for transcription. In an attempt to understand how BRCA1 exerts its function through BRCT domains, we search for partners of the BRCT domains of BRCA1. Using the yeast two-hybrid system, we identified the four and a half LIM-only protein 2 (FHL2) as a novel BRCA1 interacting protein. We demonstrate that BRCA1 and FHL2 can physically associate in vitro, in yeast, and in human cells. BRCA1 interacted with FHL2 through its second BRCT domain and the interaction of FHL2 with BRCA1 requires the last three LIM domains of FHL2. BRCA1 enhanced FHL2-mediated transcriptional activity in transient transfections. Tumor-derived transactivation-deficient BRCA1 mutants showed a reduced ability to enhance transactivation by FHL2. Lack of BRCA1 binding sites in the FHL2 completely abolished the FHL2 transactivation function. Reverse transcription polymerase chain reaction analysis showed that FHL2 mRNA levels may be downregulated in many breast cancer cell lines. These results suggest that the BRCA1–FHL2 interaction may be involved in transcriptional regulation and play a significant role in cancer cell growth.**


*Key words:* BRCA1; FHL2; Protein–protein interaction; Transactivation

## 1. Introduction

Mutations in breast cancer susceptibility gene 1 (*BRCA1*) account for approximately 40–50% of hereditary breast cancers and for nearly all familial cases with a history of both ovarian and breast cancer (for review, see [1–6]). The human *BRCA1* gene encodes a 1863-amino acid (aa) protein with a RING finger domain located in the $NH_2$-terminus and two BRCA1 C-terminal domains (BRCT1 and BRCT2) in tandem [7]. Most cancer-predisposing mutations of BRCA1 result in the deletion of the BRCT repeats. Moreover, many of the missense mutations of BRCA1 domains also lead to amino acid substitutions in the BRCT domains, underscoring the importance of the BRCT repeats for BRCA1 function in tumor suppression [8–11]. Using a chromatin unfolding assay in mammalian cells, we recently showed that BRCA1 can decondense high-order chromatin structure in the nuclei of human cells [12]. The regions in BRCA1 responsible for the high-order chromatin unfolding are mapped to the BRCT repeats.

Various lines of evidence suggest that BRCA1 is involved in multiple nuclear functions including DNA repair and transcription [13–33]. For example, BRCA1-deficient cells are hypersensitive to ionizing radiation due to defects in the repair of both oxidative DNA damage by transcription-coupled processes and double-strand breaks by homologous recombination [15,18,20,21,23]. In addition, BRCA1 functionally interacts with several repair and recombination proteins such as RAD51 [15], RAD50/MRE11/NBS1 [23], and MSH2/MSH6 [29]. BRCA1 also associates with and is phosphorylated by protein kinases that are key players in the damage checkpoint control, including ATM [26], ATR [28], CHK1 [32], and CHK2 [25].

In addition to its potential role in DNA repair, BRCA1 has also been implicated in regulation of transcription [13,14]. When fused to a heterologous DNA binding domain (DBD), the BRCA1 COOH-terminus (aa 1560–1863) including the BRCT domains has the ability to stimulate transcription. More recent work has revealed a second transactivation domain of BRCA1 that lies adjacent to the BRCT domains (aa 1293–1559) [34]. Consistent with its potential role in transcriptional regulation, BRCA1 is linked to the RNA polymerase II holoenzyme complex via RNA helicase A [17]. Furthermore, BRCA1 interacts with a variety of site-specific transcription factors or coactivators, such as p53 [19] and CBP/p300 [33].

Although there is increasing evidence suggesting that BRCA1 is involved in transcription and DNA repair, how BRCA1 exerts its function through its BRCT domains remains unclear. We report here that BRCA1 interacts with four and a half LIM-only protein 2 (FHL2) in vitro and in vivo through its second BRCT domain. FHL2 is a LIM-only protein with four and a half LIM domains [35,36]. FHL2 acts as a transcriptional activation domain when fused to a heterologous DNA -binding domain [37,38]. We further present evidence that BRCA1 enhances the transcriptional activity of FHL2.

## 2. Materials and methods

### 2.1. Plasmids

pCR3-BRCA1, pCR3-BRCA1(P1749R) and pCR3-BRCA1(Y1853-insA) were generous gifts from Dr. Wafik S. El-Deiry [39]. pcDNA3-FLAG-FHL2 was made by cloning the polymerase chain reaction (PCR)-generated full-length FHL2 cDNA from the original yeast two-hybrid library clone (pACT2-FHL2) into the *Bam*HI-*Xho*I sites

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of the pcDNA3 vector harboring a FLAG epitope sequence (pcDNA3-FLAG). Deletion mutants of FHL2 were constructed by inserting PCR-generated fragments from the corresponding cDNAs into the pcDNA3-FLAG vector. To construct pGAL4(1–94)-HA-BRCT1 and pGAL4(1–94)-HA-BRCT2, the BRCT1 and the BRCT2 domains of BRCA1 were cloned into the *Xba*I-*Bam*HI sites in the expression vector pCG-GAL4(1–94)-HA [34]. pGAL4(1–147) was generated by PCR cloning of a GAL4 DNA binding domain (DBD) fragment containing 147 amino acid residues of GAL4 DBD in pGBKT7 into the *Hin*dIII-*Bam*HI sites of pcDNA3 (Invitrogen). For generating pGAL4(1–147)-FHL2(1–279), pGAL4(1–147)-FHL2-(36–279), and pGAL4(1–147)-FHL2(1–216), the corresponding fragments were PCR-amplified and cloned into the *Bam*HI-*Eco*RV sites of the pGAL4(1–147) vector. The pGAL-LUC reporter containing four GAL4 binding sites has been described previously [34]. Constructs encoding glutathione *S*-transferase (GST) fusion proteins were prepared by amplification of each sequence by standard PCR methods, and the resulting fragments were cloned in frame into the *Bam*HI-*Xho*I sites of pGEX-KG (Amersham Pharmacia). Details of cloning are available upon request.

### 2.2. Yeast two-hybrid screen

To identify proteins that interact with the BRCT2 domain of BRCA1, the standard yeast two-hybrid screen was performed in the following manner. First, the bait plasmid was generated by inserting a PCR-amplified cDNA fragment encoding the BRCT2 sequence (aa 1756–1852) into the *Nde*I-*Eco*RI restriction sites of pAS2-1 (Clontech), resulting in an in-frame fusion with the GAL4 DBD. Second, the resulting plasmid, pAS2-BRCT2, and a human ovary cDNA prey library (Clontech) were sequentially transformed into *Saccharomyces cerevisiae* strain CG1945 according to the manufacturer's protocol (Clontech). Transformants were plated on synthetic medium lacking tryptophan, leucine and histidine but containing 1 mM 3-aminotriazole. Approximately 750 000 transformants were screened. The candidate clones were rescued from the yeast cells and reintroduced back to the same yeast strain to verify the interaction between the candidates and the BRCT2 bait. The specificity of the interaction was determined by comparing the interactions between the candidates and various bait constructs. The bait constructs, the BRCT1 domain of BRCA1 and BRCT domain of RAP1, have been described previously [12,40].

### 2.3. GST pull-down assay

GST and GST fusion proteins were expressed and purified according to the manufacturer's protocol (Amersham Pharmacia), with the induction of protein expression performed at 20°C overnight [12]. The expression vector for the FHL2 or its derivatives was used for in vitro transcription and translation using a TNT kit (Promega). The $^{35}$S-labeled proteins were incubated with 10 μg of GST derivatives bound to glutathione-Sepharose beads in 0.5 ml binding buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1 mM EDTA, 0.3 mM dithiothreitol (DTT), 0.1% NP-40 and protease inhibitor tablets from Roche). The binding reaction was performed at 4°C overnight and the beads were subsequently washed four times with the washing buffer (the same as the binding buffer). The beads were eluted in 10 μl of 2×SDS–PAGE sample buffer and protein interactions were analyzed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) followed by autoradiography.

### 2.4. Co-immunoprecipitation

293T cells were transfected using Lipofectamine 2000 (Invitrogen). Twenty-four hours after transfection, cells were washed twice with phosphate-buffered saline and lysed in 0.5 ml lysis buffer (50 mM Tris at pH 8.0, 250 mM NaCl, 0.1% NP-40, 1 mM DTT, and protease inhibitor tablets from Roche). After brief sonication, the lysate was centrifuged at 14 000 rpm for 15 min at 4°C. The supernatant was used for subsequent co-immunoprecipitation [12]. Fifteen microliters of 50% slurry of the anti-FLAG agarose beads (Sigma-Aldrich) were used in each immunoprecipitation. Immunoprecipitation was performed overnight at 4°C. The beads were centrifuged at 3000 rpm for 2 min, and washed once with the lysis buffer and three times with washing buffers, with each wash lasting at least 30 min. For determination of specificity of interaction between FHL2 and each of the BRCT domains of BRCA1, the washing buffer (50 mM Tris at pH 8.0, 500 mM NaCl, 1% NP-40, 1 mM DTT, and protease inhibitor tablets from Roche) was used. For detection of interaction between FHL2 and endogenous BRCA1, the washing buffer (50 mM Tris at pH 8.0, 250 mM NaCl, 0.5% NP-40, 1 mM DTT, and protease inhibitor tablets from Roche) was used. The precipitates were then eluted in 2×SDS–PAGE sample buffer and loaded on SDS–polyacrylamide, followed by Western blotting according to the standard procedures. Five microliters of the input crude extract were used for detecting protein expression levels. The HA-tagged BRCT domain proteins were detected using an anti-HA monoclonal antibody (Santa Cruz). The endogenous BRCA1 protein was detected using an anti-BRCA1 antibody (Oncogene).

### 2.5. Mammalian cell transfection and luciferase assay

293T cells were routinely maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% newborn calf serum at 37°C in a humidified atmosphere of 5% $CO_2$ in air. For transfection, 293T cells were plated onto 12-well plates. Twenty-four hours later, cells were transfected with plasmid constructs using Lipofectamine 2000 as instructed by the manufacturer (Invitrogen). Transfections were performed using 0.2 μg of GAL-LUC reporter, 50 ng of GAL4 DBD-FHL2 or GAL4 DBD-FHL2 mutants, and 0.5 μg expression vector for BRCA1 or its mutants. Empty vectors were used to adjust the total amount of DNA. Twenty-four hours after transfection, the cells were harvested, and luciferase and β-galactosidase activities were determined as described previously [34]. β-Galactosidase activity was used as an internal control for transfection efficiency.

### 2.6. Reverse transcription (RT)-PCR analysis

Total RNA was isolated using TRIzol Reagent according to the manufacturer's instructions (Invitrogen). First-strand cDNA was reverse transcribed from 1.0 μg total RNA with oligo(dT) primers using AMV reverse transcriptase as recommended by the supplier (Promega). One microliter of the synthesized cDNA was used for PCR amplification in a total volume of 50 μl. The oligonucleotides P1 5′-ATGACTGAGCGCTTTGACTGC-3′ and P2 5′-TCAGATGTC-TTTCCCACAGTC-3′ were used for amplification of the 840-bp coding sequence of FHL2. PCR amplifications were performed for 35 cycles using the following cycling parameters: 94°C for 1 min, 55°C for 1 min and 72°C for 1 min. The RT-PCR products were purified and ligated to a T vector, and the resulting positive clones were sequenced. The PCR for glyceraldehyde 3-phosphatase dehydrogenase (GAPDH) was performed for 25 cycles. GAPDH was used as an internal control.

### 2.7. Statistical analysis

The values are expressed as means ± S.D. Statistical significance in the luciferase activity experiments among constructs was determined by Student's *t*-test. A $P$ value $< 0.05$ was considered statistically significant.

## 3. Results

### 3.1. Identification of a BRCA1-interacting protein

Since BRCA1 is a tumor suppressor gene that is mutated in a significant fraction of cases of inherited breast and ovarian cancer, a prey library of human ovary cDNA was used in a yeast two-hybrid screen to identify proteins that interact with the BRCT2 domain of BRCA1, with the BRCT2 as bait. Out of 46 His- and LacZ-positive clones, 13 contained cDNAs with an entire open reading frame encoding the 279 amino acid residues of FHL2. The specificity of this interaction was determined by comparing the interactions between the FHL2 and various bait constructs (Fig. 1A). FHL2 failed to bind to other known BRCT domains, including the BRCT1 domain of BRCA1 and the BRCT domain of transcription factor Rap1 (Fig. 1A). Notably, all three bait constructs expressed the BRCT1 and BRCT2 of BRCA1, and BRCT of Rap1, at a comparable level in yeast cells (Fig. 1B).

### 3.2. Interaction between FHL2 and BRCA1 in vitro and in vivo

To confirm the interaction between BRCA1 and FHL2, GST pull-down experiments were performed in which in vitro

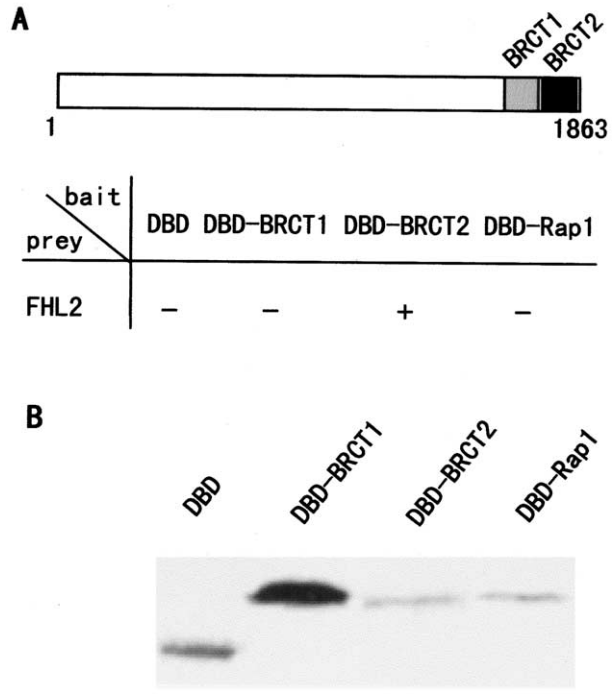


Fig. 1. Specificity of FHL2 interactions in yeast. A: Summary of the results from the yeast two-hybrid experiments. CG1945 was co-transformed with the FHL2 prey plasmid and each of the bait plasmids as indicated. Positive interaction is indicative of His- and LacZ-positive colonies. Also presented is a schematic diagram of the BRCA1 protein, illustrating the locations of BRCT domains. B: Western blotting showing the GAL4 DBD fusion protein levels in CG1945. All GAL4 DBD fusion proteins were determined by immunoblotting with an anti-GAL4 DBD antibody (Upstate).

translated [$^{35}$S]methionine-labeled FHL2 was incubated with GST-BRCT2, GST-BRCT1 or GST alone. Consistent with the two-hybrid results (Fig. 2A), FHL2 specifically bound to GST-BRCT2, but not GST-BRCT1 and GST.

To further assess the binding specificity of FHL2 to the BRCT2 region of BRCA1 in vivo, 293T cells were cotransfected with FLAG-FHL2 and HA-tagged BRCT1 or BRCT2 of BRCA1. The cell lysates were then immunoprecipitated with the anti-FLAG antibody and subsequently immunoblotted with an anti-HA antibody. Consistent with the GST pull-down results (Fig. 2B), FHL2 specifically interacted with HA-BRCT2, but not HA-BRCT1.

To determine whether FHL2 interacts with endogenous BRCA1, 293T cells were transfected with FLAG-tagged FHL2 or empty vector. FLAG-FHL2 was immunoprecipitated from cell lysates by an anti-FLAG antibody and analyzed for BRCA1 binding by Western blotting analysis. The results showed that the endogenous BRCA1 could be co-immunoprecipitated in the presence, but not in the absence, of FLAG-FHL2 (Fig. 2C). A reciprocal co-immunoprecipitation experiment using anti-BRCA1 antibodies also shows the physical interaction between BRCA1 and FHL2 (Fig. 2D).

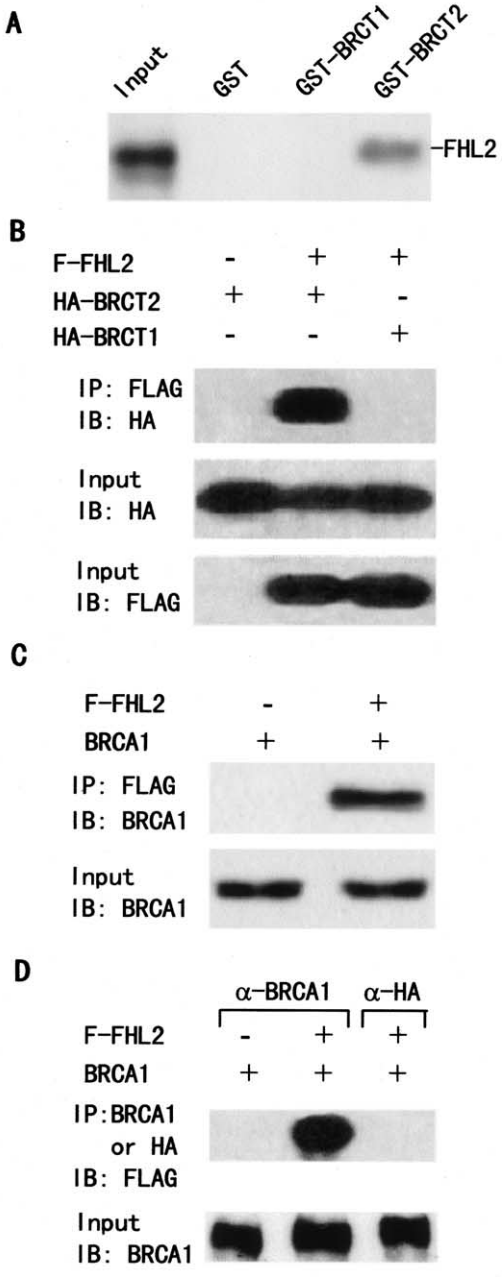


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Fig. 2. FHL2 binds to BRCA1 in vitro and in vivo. A: Interaction of full-length FHL2 with the BRCT2 domain of BRCA1 in vitro. GST pull-down assay was performed as described in Section 2. In vitro translated FHL2 was incubated with GST, GST-BRCT1, or GST-BRCT2. The bound proteins were subjected to SDS–PAGE followed by autoradiography. B: Interaction of full-length FHL2 with the BRCT2 domain of BRCA1 in vivo. 293T cells were co-transfected with the expression vectors for FLAG-tagged FHL2 and either HA-tagged BRCT1 or HA-tagged BRCT2 of BRCA1 as indicated. Lysates from the transfected cells were immunoprecipitated (IP) using anti-FLAG antibody (Sigma-Aldrich), and the immunoprecipitates were probed with an anti-HA antibody (Santa Cruz). C: Interaction of full-length FHL2 with endogenous BRCA1. 293T cells were cotransfected with the expression vector for FLAG-tagged FHL2 or empty vector as indicated. Lysates from the transfected cells were immunoprecipitated (IP) using anti-FLAG antibody (Sigma-Aldrich), and the immunoprecipitates were probed with an anti-BRCA1 antibody (Ab1; Oncogene). D: The reciprocal co-immunoprecipitation for BRCA1 and FHL2. 293T cells were transfected as in C. Lysates from the transfected cells were immunoprecipitated using either anti-BRCA1 antibodies (Ab1 and Ab3; Oncogene) or anti-HA antibody (unrelated antibody), and the immunoprecipitates were probed with an anti-FLAG antibody.

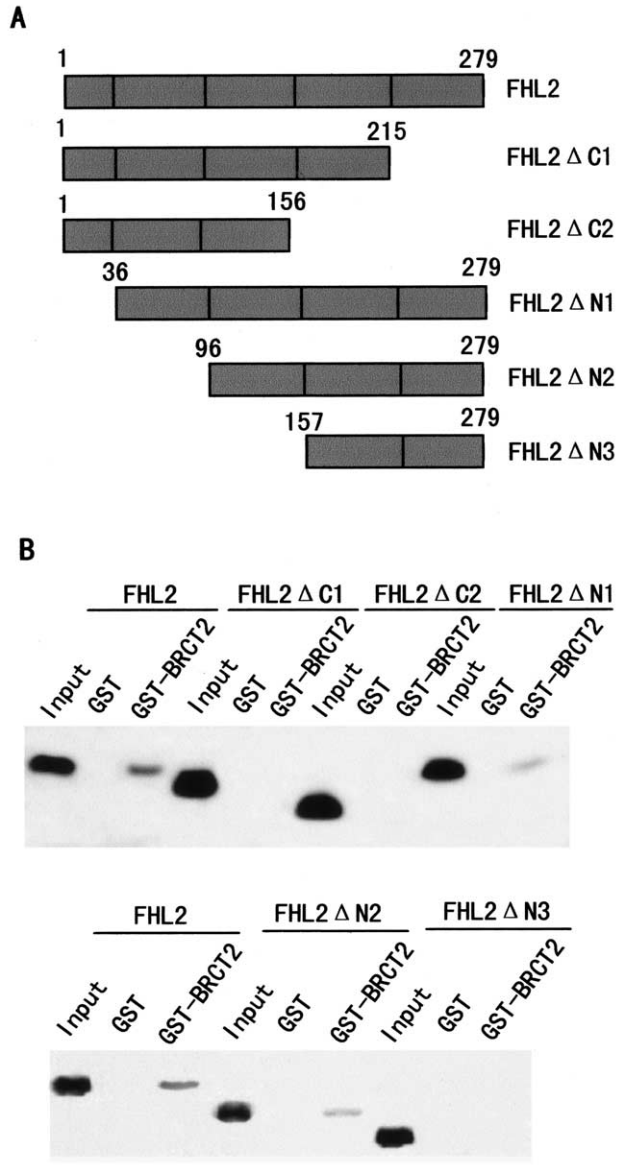


Fig. 3. Mapping of interaction regions of BRCA1 in FHL2. A: Schematic representation of the constructs used in this study. B: Mapping of the FHL2 interaction domain. GST pull-down assays were performed using $^{35}$S-labeled FHL2 or FHL2 mutants and GST-BRCT2 fusion protein. GST protein was used as a control.

### 3.3. Mapping of the BRCA1 binding domain of FHL2

To determine which region of FHL2 protein was responsible for mediating the interaction with BRCA1, a series of FHL2 deletion mutants was constructed and tested for their ability to interact with BRCT2 of BRCA1 in a GST pull-down assay (Fig. 3). The full-length or deletion mutants of FHL2 protein were generated by in vitro translation and labeled with [$^{35}$S]methionine. The glutathione-Sepharose beads loaded with similar amounts of GST or GST-BRCT2 protein were incubated with the in vitro translation products. As shown in Fig. 3B, the full-length FHL2(1–279), FHL2ΔN1 and FHL2ΔN2, but not FHL2ΔC1, FHL2ΔC2 and FHL2ΔN3, were pulled down by GST-BRCT2. However, FHL2ΔN1 and FHL2ΔN2 interacted with BRCT2 more weakly than the full-length FHL2. As negative controls, the full-length and deletion mutants of FHL2 did not associate with GST alone. These results indicated that the interaction of FHL2 with BRCA1 requires the last three LIM domains of FHL2.

### 3.4. Potentiation of the FHL2 transactivation by BRCA1

Since both BRCA1 and FHL2 have transactivation activity and BRCA1 is linked to RNA polymerase II, we sought to determine whether BRCA1 could have an effect on the activation function of FHL2. We cotransfected GAL4-LUC, GAL4 DBD-FHL2, and increasing amounts of BRCA1. As expected, FHL2 induced the transcriptional activity of the reporter 3.3-fold (Fig. 4A). BRCA1 (1.5 μg) further increased the FHL2-mediated transcriptional activity 2.4-fold. The potentiation of the FHL2 transactivation activity is dose-dependent.

To investigate whether BRCA1 transactivation function is

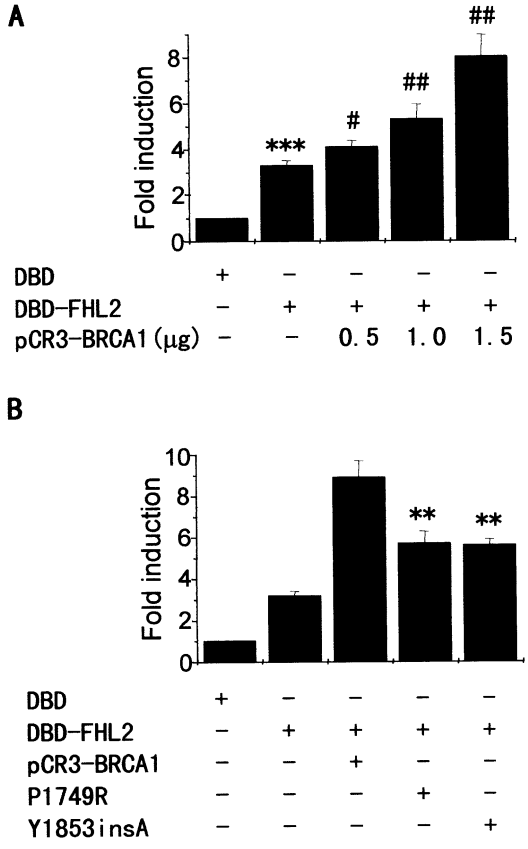


Fig. 4. BRCA1 increases FHL2 transcriptional activity. A: Effects of BRCA1 on FHL2-mediated transactivation. 293T cells were cotransfected with 0.2 μg of GAL4-LUC, 50 ng of the expression plasmid for GAL4 DBD-FHL2, and increasing amounts of the expression vector for BRCA1 as indicated. The LUC activity obtained on transfection of GAL4-LUC in the presence of GAL4 DBD alone was set as 1. Data shown are the means ± S.D. of three separate experiments. $^{***}P < 0.001$ compared to GAL4 DBD; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to GAL4 DBD-FHL2. B: Effects of transactivation-deficient BRCA1 mutants on FHL2 transcriptional activity. Cells were cotransfected with 0.2 μg of GAL4-LUC, 50 ng of the expression plasmid for GAL4 DBD-FHL2, and 1.5 μg of the expression vectors for either BRCA1 or its mutant derivatives as indicated. The relative luciferase activity in the presence of GAL4 DBD alone was set as 1. Results are expressed as means ± S.D. of three independent experiments. $^{**}P < 0.01$ compared to 293T cells cotransfected with GAL4 DBD-FHL2 and pCR3-BRCA1.

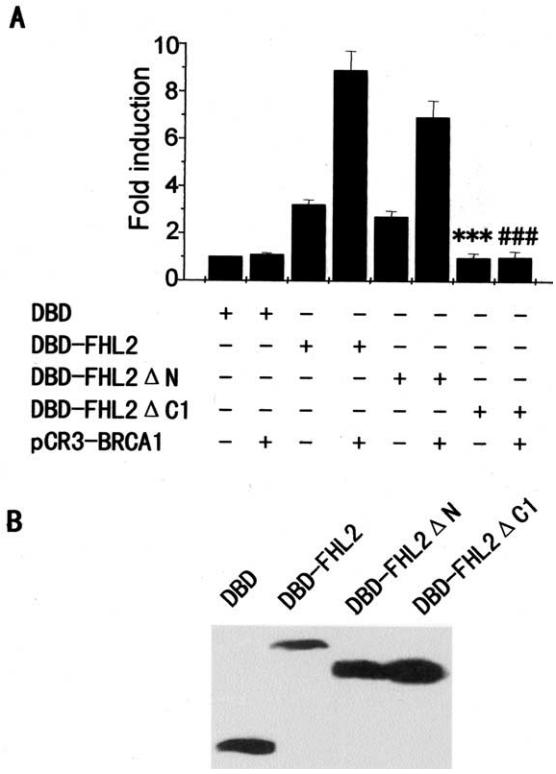


Fig. 5. Lack of BRCA1 binding sites in the FHL2 abolishes FHL2 transcriptional activity. A: Effects of mutations in FHL2 with or without BRCA1 on FHL2 transcriptional activity. 293T cells were cotransfected with 0.2 μg of GAL4-LUC, 50 ng of the expression plasmids for either GAL4 DBD-FHL2 or GAL4 DBD-FHL2 deletion mutants, with or without 1.5 μg of the expression vector for BRCA1 as indicated. The LUC activity obtained on transfection of GAL4-LUC in the presence of GAL4 DBD alone was set as 1. Data are the means ± S.D. of three independent experiments. $^{***}P < 0.001$ compared to GAL4 DBD-FHL2; $^{###}P < 0.001$ compared to 293T cells cotransfected with GAL4 DBD-FHL2 and pCR3-BRCA1. B: Western blotting showing expression of the GAL4 derivatives. Cells were transfected as in A. Whole-cell extracts were prepared, and equivalent amounts of each extract were probed with anti-GAL4 DBD antibody (Upstate).

required for regulation of FHL2 transactivation activity, we tested two previously described tumor-derived transactivation-deficient BRCA1 mutants, P1749R and Y1853insA [39], in the transient transfection experiments. As shown in Fig. 4B, the two mutants could reduce the enhancement of FHL2 transactivation, suggesting that transactivation by BRCA1 may contribute to the maximal FHL2 transactivation function.

### 3.5. Lack of BRCA1 binding sites in the FHL2 abolishes FHL2 transactivation function

To test the possibility that BRCA1–FHL2 interaction is required for the FHL2 transactivation, two FHL2 deletion mutants, GAL4 DBD-FHL2ΔN and GAL4 DBD-FHL2ΔC1, were made. In the former deletion mutant, the N-terminal half LIM domain (aa 1–35) was deleted, and in the latter deletion mutant, the C-terminal last LIM domain (aa 217–219) was deleted. 293T cells were cotransfected with the GAL4-LUC reporter, expression vectors for either GAL4 DBD-FHL2, GAL4 DBD-FHL2ΔN, or GAL4 DBD-FHL2ΔC1. As shown in Fig. 5, the GAL4 DBD-FHL2ΔN containing the BRCA1 binding sites still retained the FHL2 transcriptional activity, whereas the GAL4 DBD-FHL2ΔC1 mutant lacking the BRCA1 binding sites completely abolished the FHL2 transcriptional activity. This is not attributable to decreased expression of the GAL4 DBD-FHL2ΔC1 mutant. In contrast, the GAL4 DBD-FHL2ΔC1 mutant was expressed at higher levels than wild-type GAL4 DBD-FHL2 and the GAL4 DBD-FHL2ΔN. In addition, BRCA1 did not rescue the transcriptional defect of the GAL4 DBD-FHL2ΔC1 mutant. Taken together, these findings suggest that the BRCA1–FHL2 interaction contributes to the transactivation function of FHL2.

### 3.6. Expression of FHL2 mRNA in breast cancer cell lines

Human FHL2 was originally identified by subtractive cloning as a LIM-only protein downregulated in rhabdomyosarcoma [36]. FHL2 mRNA was shown to be detectable in many tissues [36,41]. FHL2 appeared to be highly expressed in heart and ovary; moderate levels could be detected in placenta, uterus, mammary gland, and adrenal gland; and low levels seemed to exist in skeletal muscle, colon, bladder, prostate, stomach, trachea, testis, small intestine, thyroid gland, and kidney. FHL2 mRNA was also found to be expressed in various cancer cell lines, such as breast cancer, leukemia, cervical cancer, colon cancer, and lung cancer cell lines. Since only a limited number of breast cancer cell lines (T47D, MCF7, HBL100 and MDA-MB-231) were tested for FHL2 mRNA expression, we asked whether FHL2 mRNA is widely expressed in breast cancer cell lines. We prepared mRNA from eight breast cancer cell lines and an immortalized normal breast epithelial cell line, MCF-10A, and performed RT-PCR using primers for amplification of full-length FHL2 (Fig. 6). As previously reported [42], FHL2 mRNA was expressed in T47D cells. The identity of FHL2 detected was further confirmed by DNA sequencing. Although our assay was only semi-quantitative, comparison to the GAPDH signal allowed some estimations about the relative FHL2 expression in different cell lines. Compared with the normal breast epithelial cell line MCF-10A, all breast cancer cell lines tested except T47D expressed FHL2 mRNA at lower levels, suggesting that FHL2 may play an important role in breast cancer development.

## 4. Discussion

Here we present evidence of physical and functional interactions between BRCA1 and FHL2. The physical interaction has been validated by a number of in vitro and in vivo assays, including yeast two-hybrid, in vitro GST pull-down, and in

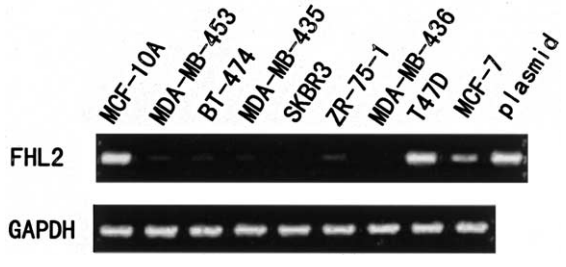


Fig. 6. FHL2 mRNA expression in breast cancer cell lines. RT-PCR from the selected cell lines was performed as described in Section 2. GAPDH was used as an internal control. The pcDNA3-FLAG-FHL2 plasmid (0.5 pg) served as a positive control.

vivo co-immunoprecipitation. Importantly, we can demonstrate that BRCA1 functionally enhances FHL2 transactivation, suggesting that FHL2 is a downstream target of BRCA1.

The BRCT domain, first identified in the BRCA1 C-terminal region, is an approximately 95-aa region defined by distinct hydrophobic clusters of amino acids [43]. This domain is considered to be a protein–protein interaction module, which can either bind different BRCT domains specifically or interact with other unknown protein folds. We have previously shown that BRCA1 interacts with a novel COBRA1 protein through its first BRCT domain [12]. The observation that the LIM-only protein FHL2 specifically interacted with the BRCT2 domain of BRCA1, but not other BRCT domains, suggests that the BRCT motif may only provide the architectural basis for binding to FHL2. Additional amino acid residues in the BRCT region that are unique to the BRCT2 of BRCA1 may serve as the actual contact points for the LIM domains of FHL2.

The transcription of protein-encoding genes in eukaryotic cells requires RNA polymerase II and a set of general transcription factors that include the TATA binding protein, TFIIB, TFIIE, TFIIF and TFIIH [44]. These factors assemble at the core promoter and are sufficient for accurate transcription initiation in vitro. BRCA1 and FHL2 were found to carry an intrinsic activation function [13,14,37]. Immunoprecipitation of BRCA1 complexes specifically purifies transcriptionally active RNA polymerase II holoenzyme and transcription factors TFIIE, TFIIF and TFIIH [16]. The fact that BRCA1 enhanced the transcriptional activity of FHL2 suggests that the interaction between BRCA1 and FHL2 may facilitate the recruitment of RNA polymerase II holoenzyme and other general transcription factors to promoter regions. The findings that lack of BRCA1 binding sites in the FHL2 abolished the FHL2 transactivation function and that tumor-derived transactivation-deficient BRCA1 mutants impaired FHL2 transcriptional enhancement indicate that the transcriptional activity of BRCA1 contributes to the transcriptional activity of FHL2. It will be interesting to investigate how the BRCA1–FHL2 interaction affects transcriptional regulation.

In this study, we used a GAL4-responsive reporter to assess the effect of BRCA1 on FHL2 transcriptional activity. Use of this reporter instead of a BRCA1- or FHL2-responsive reporter circumvents some of the issues regarding BRCA1- or FHL2-specific responsive sequences. BRCA1 has been reported to interact with the activator protein 1 (AP-1) transcription factor JunB using a yeast two-hybrid screen [45]. JunB enhances the transcriptional activity of BRCA1 fused to the GAL4 DBD from a GAL4 responsive reporter. Using a yeast two-hybrid screen, the tumor suppressor menin was found to interact with the AP-1 transcription factor JunD [46]. Menin represses the transactivation by JunD fused to the GAL4 DBD from a GAL4-responsive reporter. Here we show that BRCA1 enhances FHL2-mediated transcriptional activity using a GAL4-responsive reporter. Both BRCA1 and FHL2 have been shown to bind and activate the transcriptional activity of androgen receptor (AR) in an androgen-dependent manner [37]. However, we failed to demonstrate that BRCA1 and FHL2 synergistically enhanced AR-dependent transactivation activity using an androgen response element-containing luciferase reporter (data not shown), suggesting that BRCA1, FHL2 and AR may not exist in the same complex or that the synergistic effects of BRCA1 and FHL2 may be promoter- or cell type-specific. Although BRCA1 was found to activate the cyclin-dependent kinase inhibitor p21-promoter luciferase reporter [39], the BRCA1-FHL2 interaction does not have any effect on the p21-promoter reporter gene expression (data not shown). Further studies are required to determine downstream target gene expression regulated by the BRCA1–FHL2 interaction.

FHL2 expression was originally shown to be heart-specific. However, many recent studies indicate that FHL2 is not restricted because it can be expressed in ovary, placenta, uterus, mammary gland, and adrenal gland. We report here that FHL2 mRNA expression may be downregulated in many breast cancer cell lines. Since both BRCA1 and FHL2 are capable of efficiently inducing apoptosis [41,47–49], it is possible that the BRCA1–FHL2 interaction may be involved in regulation of cancer cell growth.

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