The BRCA1 tumor suppressor gene has previously been implicated in induction of high levels of apoptosis in osteosarcoma cell lines. Overexpression of BRCA1 was shown to induce an apoptotic signaling pathway involving the c-Jun N-terminal kinase (JNK), but the signaling steps upstream and downstream of JNK were not delineated. To better understand the role of BRCA1 in apoptosis, we examined the effect of wild-type and C-terminal-truncated dominant negative BRCA1 on breast and ovarian cancer cell lines subjected to a number of different pro-apoptotic stimuli, including growth factor withdrawal, substratum detachment, ionizing radiation, and treatment with anticancer agents. All of these treatments were found to induce substantial levels of apoptosis in the presence of wild-type BRCA1, whereas dominant negative BRCA1 truncation mutants diminished the apoptotic response. Subsequent mapping of the apoptotic pathway induced by growth factor withdrawal demonstrated that BRCA1 enhanced signaling through a pathway that sequentially involved H-Ras, MEKK4, JNK, Fas ligand/Fas interactions, and caspase-9 activation. In addition, the pathway functioned independently of the p53 tumor suppressor. These data suggest that BRCA1 is an important modulator of the response to cellular stress and that loss of this apoptotic potential due to BRCA1 mutations may contribute to tumor development.

Mutations in the BRCA1 tumor suppressor gene are found in many families with inherited breast and ovarian cancers and about half of families with a history of breast cancer only (1–3). BRCA1 encodes an 1863-amino acid protein (1) that is located predominantly in the nucleus (4–6). This polypeptide has been implicated in the regulation of a wide variety of biological functions, including growth suppression, induction of apoptosis, cell cycle regulation, response to DNA damage, and maintenance of genome stability (7–11).

BRCA1 contains several well-defined functional domains. An N-terminal RING finger domain interacts with BARD1 (12), E2F transcription factor family members, cyclins and cyclin-dependent kinases (13). A domain in the middle of BRCA1 associates with the DNA repair protein RAD51 (10). The C-terminal BRCT domains are involved in transcription activation, growth inhibition and tumor suppression through interactions with RNA helicases, RNA polymerase II, TFIIH, TFIIE, BRCA2, and RAD51 (7, 14–18).

Several observations also support a role for BRCA1 in regulation of transcription. BRCA1 activates the p21WAF1/Cip1 promoter in cells that contain wild-type or mutant p53 (19), suggesting that one of the mechanisms by which BRCA1 regulates cell cycle and suppresses growth is through the induction of p21. Additionally, BRCA1 binds the CIP transcriptional repressor that inhibits BRCA1-mediated activation of the p21WAF1/Cip1 promoter (20), interacts with CBP/p300 (21), and interacts with STAT1 to induce expression of the γ-interferon gene (IFNγ) (22). These data imply that BRCA1 might be directly involved in transcriptional activation of specific genes.

A growing body of evidence has also implicated BRCA1 in the preservation of genome integrity. Initial studies demonstrated that BRCA1 binds to the RAD50 and RAD51 DNA repair proteins (10, 23). More recent studies have implicated BRCA1 in transcription-coupled repair (24) and double strand DNA break repair (25). In accord with a possible role for BRCA1 in repair (or the control of repair), breast tumors from patients with BRCA1 germ-line mutations contain 2- to 3-fold more chromosomal rearrangements than sporadic cancers.

Consistent with the proposed role of BRCA1 as a tumor suppressor, it has been observed that BRCA1 inhibits breast and ovarian cancer cell proliferation in vitro and in an experimental tumor model (7, 26). Conversely, selective reduction of BRCA1 mRNA levels using antisense RNA also induces more rapid cell growth and promotes cell transformation in NIH 3T3 fibroblasts (7). Whether the effects of BRCA1 on transcription and genome maintenance are sufficient to explain these results have been unclear. However, several recent reports have suggested that BRCA1 might also play a role in induction of apoptosis. Shao and colleagues (8) reported that expression of BRCA1 in mouse fibroblast and human breast cancer cell lines resulted in apoptosis in response to serum deprivation or calcium ionophore treatment. BRCA1-expressing prostate cancer DU-145 cells were subsequently shown to be susceptible to drug-induced apoptosis (27). More recently, Harkin and colleagues (28) demonstrated that BRCA1 expression can induce...
apoptosis through activation of a c-Jun N-terminal kinase/stress-activated protein kinase (JNK/SAPK). In the present study, we have further delineated the relationship between BRCA1 and JNK-dependent apoptotic signaling in breast and ovarian cancer cell lines. We provide evidence that BRCA1 modulates stress-induced apoptotic signaling through a pathway that sequentially involves the H-Ras proto-oncogene, MEKK4, JNK, Fas (CD95)/FasL interactions, and activation of procaspase-8. In addition, we present evidence for dominant negative activity of BRCA1 mutants in the context of this apoptotic response.

EXPERIMENTAL PROCEDURES

Materials—Immunological reagents were purchased from the following suppliers: BRCA1 rabbit polyclonal antibodies directed to the C and N termini of BRCA1 (66056E and 66036E) from PharMingen; antibodies against caspase-8, caspase-9, Fas, Fas-L, phospho-JNK, and phospho-MEKK 1–4 from Santa Cruz Biotechnology; inhibitory anti-Fas antibodies ZB4 and Nok2 from Kamiya and PharMingen, respectively; p53 antibody from Oncogene Research Products; and alkaline phosphatase-conjugated secondary antibody from the Jackson ImmunoResearch Laboratories; z-VAD(OMe)-fmk was obtained from Enzyme Systems Products (Dublin, CA). An Annexin V apoptosis detection kit was purchased from R & D Systems (Minneapolis, MN). Propidium iodide (PI) and paclitaxel were procured from Sigma. An enhanced chemiluminescence kit was purchased from Roche Molecular Biochemicals.

Plasmids—The full-length BRCA1 coding sequence was subcloned into the pCR3.1 (Invitrogen) mammalian expression vector. Two truncation mutants of BRCA1 (BRCA1D5382-insC and BRCA1D5677-insA) were generated by polymerase chain reaction as described previously (19, 26). The GFP-encoding plasmid pEGFP-N-1 was from CLONTECH. MC159 and CrmA expression constructs were kindly provided by J. Bertin (National Institutes of Health) and C. Young (Mayo Clinic), respectively. Dominant negative expression plasmids dnMEKK1-HA-pCEPT4, dnMKK4-pRSET(D55–72), and dnMKK7-FLAG-pcDNA3 were provided by David McKean (Mayo Clinic). The dnJNK(APF)-pcDNA3 was kindly provided by Roger Davis. The Ras-N17 mutant construct was provided by Larry Karnitz (Mayo Clinic).

Cell Culture and Transfection—The human breast adenocarcinoma cell lines MCF7 and T47D were obtained from ATCC (Manassas, VA). The ovarian cancer cell line OV177 was obtained from Cheryl Conover. The MCF7, T47D, and OV177 cells were grown in Dulbecco’s modified essential medium, RPMI 1640, and α-minimal essential medium, respectively. Each of these was supplemented with 10% bovine calf serum, 100 units/ml penicillin G, 100 μg/ml streptomycin, and 2 mM glutamine. Mouse embryo fibroblast (MEF) p53+/− and MCF7 HPV16 cells were grown in Dulbecco’s modified essential medium with 10% fetal bovine serum and the additives listed above. Cells were plated (1 × 10^6 cells) into 100-mm dishes,
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Figure 2. Truncated BRCA1 exerts an anti-apoptotic effect. A, expression of the BRCA1Δ5382-insC construct results in formation of a stable truncated BRCA1 protein. BRCA1 wild-type (wt) and the BRCA1Δ5382insC mutant were transiently expressed in MCF7, T47D, and OV177 cells. Lysates were harvested 24 h after transfection. Aliquots containing 100 μg of protein from each cell line were probed using N-terminal BRCA1 (60036E) antibody. The wild-type BRCA1 protein is approximately 220 kDa and the C-terminal-truncated mutant BRCA1Δ5382-insC is 180 kDa in size. B, the BRCA1Δ5382-insC mutant inhibits BRCA1-dependent apoptosis. After transient co-transfection of full-length BRCA1 or the C-terminal-truncated BRCA1Δ5382-insC with a GFP plasmid, GFP-expressing MCF7 cells were incubated for 24 h in the presence and absence of serum, fixed, stained with PI and Annexin V, and analyzed by flow cytometry.

RESULTS

Ectopic Expression of BRCA1 Facilitates Apoptosis in Breast and Ovarian Cancer Cell Lines—To study the functional properties of BRCA1, we transiently overexpressed full-length BRCA1 cDNA or pCR3.1 empty vector in two breast (MCF7 and T47D) and one ovarian (OV177) cancer cell line. Western blot analysis using the C-terminal anti-BRCA1 (66046E) antibody showed a 7- to 8-fold increase in BRCA1 protein on BRCA1 transfection when compared with the endogenous BRCA1 level in all three cell lines used in this study (Fig. 1A). To evaluate the effect of BRCA1 overexpression on cellular apoptosis, we cotransfected cells with BRCA1 or pCR3.1 and GFP, enriched for transfected cells by sorting for GFP-positive cells, incubated the transfected cells under various conditions for an additional 24 h, and then measured apoptosis using Annexin V and PI. Cells exposed to normal growth conditions (Fig. 1B, +Serum) demonstrated a 6- to 7-fold increase in Annexin V positivity when transfected with BRCA1 as compared with empty vector. For example, 17 ± 2% of BRCA1-transfected MCF7 cells were apoptotic, whereas only 2.5 ± 1% of vector-transfected MCF7 cells were apoptotic under the same conditions. These results suggest that increased levels of wild-type BRCA1 increase the rate of spontaneous apoptosis in the three cell lines.

To further examine the effect of BRCA1 expression on cellular apoptosis, cells were exposed to various apoptotic stimuli, including serum withdrawal, γ-irradiation, and treatment with paclitaxel. Staining with Annexin V and PI revealed that 13 ± 1%, 17 ± 2%, and 11 ± 1% of the vector-transfected MCF7, T47D, and OV177 cells, respectively, were apoptotic after removal of serum. In contrast, 42 ± 4%, 53 ± 4%, and 38 ± 3% of the BRCA1-transfected MCF7, T47D, and OV177 cells were apoptotic after serum withdrawal (Fig. 1B). Similarly, forced overexpression of BRCA1 enhanced the amount of apoptosis observed after treatment with γ-irradiation (Fig. 1C) and paclitaxel (Fig. 1D). These results suggest that expression of BRCA1 facilitates the response of breast and ovarian cancer cells to a variety of apoptotic stimuli.

Wild-type BRCA1 Is Required for Apoptosis—To further eva-
uate the role of BRCA1 in apoptosis, we transiently transfected the wild-type BRCA1 and the pCR3.1 vector into HCC1937 cells, which express only the 5382insC-truncated form of BRCA1. When these cells were incubated in the presence and absence of serum for 24 h, the pCR3.1-transfected cells displayed only minor levels of apoptosis (<3%) (Fig. 1E). In contrast, ectopic expression of BRCA1 at levels that exceeded the expression level of the endogenous BRCA1 protein (inset, Fig. 1E) increased the rate of apoptosis in the presence of serum to 10%, and to 38% upon serum withdrawal (Fig. 1E). The low levels of spontaneous and serum withdrawal-induced apoptosis in the absence of full-length BRCA1 suggest that BRCA1 plays an important role in this apoptotic process.

**BRCA1 Mutants Abolish BRCA1-dependent Apoptosis**—To further explore the role of BRCA1 in cellular apoptosis, we transiently transfected the wild-type BRCA1 and the pCR3.1 vector into HCC1937 cells, which express only the 5382insC-truncated form of BRCA1. When these cells were incubated in the presence and absence of serum for 24 h, the pCR3.1-transfected cells displayed only minor levels of apoptosis (<3%) (Fig. 1E). In contrast, ectopic expression of BRCA1 at levels that exceeded the expression level of the endogenous BRCA1 protein (inset, Fig. 1E) increased the rate of apoptosis in the presence of serum to 10%, and to 38% upon serum withdrawal (Fig. 1E). The low levels of spontaneous and serum withdrawal-induced apoptosis in the absence of full-length BRCA1 suggest that BRCA1 plays an important role in this apoptotic process.

**BRCA1 Facilitates Serum Withdrawal-induced Apoptosis by Enhancing Signaling through a Ras, MEKK4, and JNK Pathway**—Harkin et al. (28) recently demonstrated that BRCA1-induced apoptosis is associated with activation of JNK/SAPK. However, signaling pathways upstream of JNK/SAPK, or the
downstream targets of JNK/SAPK, were not identified. To assess the role of JNK signaling in BRCA1-dependent serum starvation-induced apoptosis, we investigated the involvement of other components of MAPK signaling pathways. MCF7 and OV177 cells were transfected with pCR3.1 vector, full-length BRCA1 or BRCA1Δ5382-insC, sorted, and cultured in the presence and absence of serum for 24 h. Cell lysates prepared from these cells were subjected to immunoblotting with antisera that recognize phosphorylated species of MEKK1, MEKK4, and JNK. BRCA1 overexpression did not alter MEKK1 phosphorylation (data not shown) but did result in increased levels of phospho-MEKK4 and phospho-JNK, which were increased even further after growth factor withdrawal (Fig. 3, A and B). Conversely, the BRCA1Δ5382-insC mutant, which failed to induce apoptosis (Fig. 2B), failed to induce phosphorylation of these proteins (Fig. 3, A and B). Further evidence that activation of these kinases plays a critical role in BRCA1-dependent serum withdrawal-triggered apoptosis came from the observation that expression of dn-MKK4 or dn-JNK completely blocked BRCA1-dependent apoptosis in both MCF7 and OV177 cells in the presence and absence of serum (Figs. 3, C–E). In contrast, dominant negative forms of MEKK1 and MKK7 did not affect BRCA1-dependent apoptosis either in the presence or absence of serum (Fig. 3, C and D). These results suggest that JNK and its upstream activators MKK4 and MEKK4 are required for BRCA1-dependent apoptosis after serum withdrawal.

A number of activators of MEKK proteins have been identified previously, including Ha-Ras (31). Although Ras appears to play a major role in cell survival and proliferation, a role in induction of apoptosis has also been reported (32, 33). To investigate the role of Ras in BRCA1-dependent growth factor withdrawal-induced apoptosis, MCF7 and OV177 cells were transiently co-transfected with BRCA1 and a dominant negative form of H-Ras (Ras N17). Expression of Ras N17 resulted in complete abrogation of BRCA1-dependent apoptosis in the presence and absence of serum starvation (data not shown). Collectively, these observations suggest that BRCA1 facilitates apoptotic signaling through a Ras/MEKK4/MKK4/JNK pathway.

Fas (CD95/APO-1) Mediates BRCA1-dependent Serum Withdrawal-induced Apoptosis—Recent reports have suggested a role for Fas and FasL in neuronal apoptosis following nerve growth factor withdrawal (34). In addition, JNK has been shown to up-regulate the FasL promoter by activation of the c-Jun and ATF2 transcription factors (35). When combined with the results from the experiments described above, these observations suggest that Fas and FasL play a role in BRCA1-dependent apoptosis in response to growth factor withdrawal. To evaluate this hypothesis, MCF7 and OV177 cells were transiently transfected with pCR3.1, full-length BRCA1, or BRCA1Δ5382-insC, sorted, incubated in the presence and absence of serum for 24 h, and immunoblotted for Fas and FasL. BRCA1 expression up-regulated both Fas and FasL in MCF7 and OV177 cells (Fig. 4, A and B), and the levels of these proteins were further enhanced by serum withdrawal (Fig. 4, A and B). Conversely, BRCA1Δ5382-insC completely blocked induction of Fas and FasL by endogenous and ectopically expressed Fas and FasL in breast and ovarian cancer cells after serum withdrawal. A and B, analysis of Fas (A) and FasL (B) protein levels in MCF7 and OV177 cells transfected with GFP and pCR3.1 vector, wild-type BRCA1, or BRCA1Δ5382-insC. After GFP-expressing cells were incubated in the presence and absence of serum for 24 h, 30 μg of cell lysate was immunoblotted with anti-Fas antibody (A) or anti-FasL antibody (B). To confirm equivalent loading, blots were probed with anti-Histone H2B. C and D, inhibition of Fas signaling abolishes BRCA1-dependent serum withdrawal-induced apoptosis. MCF7 cells (C) or OV177 cells (D) were transiently transfected with GFP and BRCA1 or pCR3.1 in the absence or presence of plasmid encoding dnFADD or MC159. GFP-expressing cells were incubated for 24 h with Nok2 and ZB4 in the absence or presence of serum. Annexin V-positive/PI-negative cells were quantitated by flow cytometry.
pressed BRCA1 in both cell lines (Fig. 4, A and B).

To further evaluate the role of Fas, Fas-L, and the adaptor protein FADD in this apoptotic signaling pathway, MCF7 and OV177 cells transfected with BRCA1 and the vector control were incubated with ZB4 or Nok2 in the presence and absence of serum. The Nok2 and ZB4 monoclonal antibodies bind to the Fas receptor and prevent cross-linking by Fas-L. Both of these antibodies abolished BRCA1-induced apoptosis in the presence and absence of serum (Fig. 4C). Likewise, co-expression of a dominant negative version of the adaptor protein FADD, or MC159, a Molluscum contagiosum viral inhibitor of FADD signaling (36), with BRCA1 abolished BRCA1-induced apoptosis (Fig. 4C). These results suggest that the BRCA1-dependent apoptosis in response to serum deprivation is Fas-dependent and that mutant BRCA1 blocks this apoptotic signaling pathway in both breast and ovarian cancer cells.

**BRCA1-induced Apoptosis Is Caspase-dependent**—Two pathways of Fas-dependent caspase activation have been described (37, 38). One involves recruitment of caspase-8 followed by direct activation of effector caspases, whereas the second involves activation of caspase-8 and cleavage of the Bcl-2 family member Bid to yield a fragment that induces release of cytochrome c from mitochondria and subsequent activation of effector caspases through the Apaf-1/caspase-9 pathway (39). To assess the activation of caspase-8 and caspase-9 in response to BRCA1 overexpression, lysates from MCF7 and OV177 cells transfected with pCR3.1, wild-type BRCA1, or BRCA1Δ5382-insC were immunoblotted with antisera directed to the cleaved, active forms of caspase-8 and caspase-9. BRCA1 overexpression resulted in cleavage of both caspase-8 and caspase-9 (Fig. 5, A and B). Serum starvation for 24 h resulted in further activation of both caspase-8 and caspase-9 (Fig. 5, A and B). As expected, the activation of caspase-8 and caspase-9 was completely blocked by co-expression of BRCA1Δ5382-insC (Fig. 5, A and B). In addition, the z-VAD.fmk general caspase inhibitor inhibited all BRCA1-dependent apoptosis. These results suggest that caspase-8 and caspase-9 are both activated during BRCA1-dependent apoptosis.

To further assess the role of these initiator caspases, we co-expressed CrmA, which selectively inhibits caspase-1 and caspase-8 (40), and a dominant negative caspase-9 construct, which inhibits caspase-9 activation (41) with BRCA1 and pCR3.1 vector in MCF7 and OV177 cells. Both CrmA and dn-caspase-9 abrogated BRCA1-induced apoptosis (Fig. 5, C and D), suggesting that the BRCA1-dependent signal is transduced through a caspase-9/Bid/Apaf-1/cytochrome c/caspase-9 (type II) pathway in both cell lines.

**The MAPK Pathway Is Upstream of Fas Activation in the...**
The MAPK signaling pathway is upstream of the death receptor and caspase signaling pathway in BRCA1-dependent, serum withdrawal-induced apoptosis. A, inhibition of MKK4 and JNK signaling abrogates Fas and FasL induction and caspase-8 and caspase-9 activation. MCF7 cells were cotransfected with GFP and dnM KK4 or dnJNK along with wild-type BRCA1 or pCR3.1 vector. Cells were sorted for GFP and incubated in the presence and absence of serum for 24 h. Cell lysates were immunoblotted with antibodies against Fas and FasL and caspase-8 and active caspase-9. B, inhibition of Fas signaling and caspase-8 function does not affect JNK activation. MCF7 cells co-transfected with GFP and dn-FADD or CrmA, along with BRCA1 wild-type or pCR3.1 vector, were sorted for GFP, and incubated in the presence or absence of serum for 24 h. Immunoblots of lysates with anti-phospho-JNK antibody were used to assess the level of JNK activation.

**DISCUSSION**

The results of the present study demonstrate that BRCA1 enhances apoptosis induced by other stimuli in breast and ovarian cancer cell lines; that a truncation mutant lacking the C-terminus of BRCA1 suppresses apoptosis, including spontaneous apoptosis, in these cell lines; that BRCA1-dependent apoptosis occurring after serum withdrawal proceeds through a H-Ras/MEKK4/JNK signaling pathway followed by increased expression of Fas and FasL and by activation of caspase-8; and that this Fas-dependent signaling pathway is independent of p53 function. Each of these observations has potentially important implications for current understanding of the role of BRCA1 as a tumor suppressor protein.

A previous study indicated that BRCA1 overexpression can result in induction of high levels of spontaneous apoptosis in U2OS osteosarcoma cells (28). In the present study, overexpression of BRCA1 in breast and ovarian cancer cells was associated with much more modest levels of apoptosis, with 10–12% of the cells undergoing apoptosis in the absence of apoptotic stimuli. The difference between our results and those obtained previously might reflect differences in cell type or in the degree of BRCA1 overexpression. However, the lower levels of apoptosis observed after BRCA1 transfection allowed us to examine the effect of BRCA1 on sensitivity of cells to other apoptotic stimuli. Results of these studies demonstrated that BRCA1 overexpression enhanced the apoptotic response to a variety of stimuli, including withdrawal of serum-derived survival factors, exposure to ionizing radiation, or treatment with the chemotherapeutic agent paclitaxel. These observations suggest that BRCA1 is capable of modulating the apoptotic response to a variety of stimuli.

To further evaluate the effects of BRCA1 on apoptosis, we examined BRCA1 null HCC1937 cells (46). Levels of apoptosis remained low in these cells even after serum starvation, but increased substantially upon ectopic expression of wild-type BRCA1. Conversely, expression of stoichiometric amounts of certain BRCA1 truncation mutants was shown to decrease spontaneous and serum withdrawal-induced apoptosis in cells expressing wild-type BRCA1. These observations not only help establish a role for endogenous BRCA1 in the apoptotic re-
and ovarian cancer cells is p53 independent. A papilloma virus 16 analysis of BRCA1 expression in MCF7 cells stably expressing human BRCA1 (66036E) antibody. BRCA1 migrated as a 220-kDa band. The pCR3.1 vector, wild-type BRCA1, or BRCA1 expressing HPV16 E6, or vector control, were transiently transfected with enhancer after serum deprivation in the absence of functional The percentage of subdiploid cells is shown. The presence and absence of serum for 24 h, fixed, and stained with PI. In the presence and absence of serum for 24 h. The knock-down of Bax and GADD45, GADD143, and IFN-g, as well as repression of several genes, including cyclin B1 (19, 22, 26, 28, 51, 54), it is possible that these or other transcriptional targets of BRCA1 might regulate the apoptotic process described in this study. Harkin and colleagues (28) reported that BRCA1-induced apoptosis in U2OS osteosarcoma cells is associated with JNK activation. However, the apoptotic signaling pathways upstream of JNK in this model system were not reported. Likewise, the signaling pathways upstream of JNK in nerve growth factor withdrawal-induced apoptosis in neuronal PC12 (34) and in detachment-associated apoptosis (anoikis) in various cell types (55, 56) have not been well defined. In the present study, we investigated the signal transduction pathway that was activated in a BRCA1-dependent manner by serum withdrawal. Results of this analysis identified a pathway involving activation of MEKK4 and JNK by phosphorylation. This pathway was inhibited by Ras N17, dn-MKK4, dn-MEKK4, and dn-JNK, pointing to a pathway that involves sequential signaling from H-Ras to MEKK4, M KK4, and JNK. Although the apparent involvement of H-Ras in this pathway was somewhat unexpected, a number of studies have recently shown that Ras proteins can regulate apoptotic responses in a cell type- and stimulus-dependent fashion (33). In particular, it has been shown that Ras can induce apoptosis by binding and activating MEKK1 (31). Other studies have refined this model by demonstrating that Ras induces apoptosis through a Rac1- and p21-activated kinase-dependent pathway (57), and through a Ras/Rac1/CDC42/MLK3 (mixed lineage kinase 3)/MEKK pathway (58). Most recently, the Ras-associated apoptotic pathway has been shown to signal through JNK in a p53-independent manner (59) similar to the pathway identified above. In the present study we did not attempt to define the specific signaling pathway upstream of H-Ras, nor did we determine how BRCA1 is modulating signaling through this pathway. These are areas for future investigation. Although BRCA1 was shown to activate JNK in U2OS cells (28), the pathways downstream of JNK in BRCA1-dependent apoptosis were not reported. Previous studies have raised the possibility that JNK can function as a downstream potentiator of BRCA1-dependent apoptosis in serum-deprived breast and ovarian cancer cells is p53 independent. A, immunoblot analysis of BRCA1 expression in MCF7 cells stably expressing human papilloma virus 16 E6 gene (HPV16 E6) following transient transfection with pCR3.1 vector, wild-type BRCA1, and BRCA15382-insC. Aliquots containing 100 µg of protein were probed with the N-terminal BRCA1 (66036E) antibody. BRCA1 migrated as a 220-kDa band. The BRCA15382-insC mutant encodes a 180-kDa polypeptide. B, BRCA1 enhances apoptosis after serum deprivation in the absence of functional p53. p53 null mouse embryo fibroblasts (MEFs) and MCF7 cells expressing HPV16 E6, or vector control, were transiently transfected with pCR3.1 vector, wild-type BRCA1, or BRCA15382-insC mutant, along with GFP. The GFP-positive cells were isolated by FACS, incubated for 24 h, fixed, and stained with PI. The percentage of subdiploid cells is shown. C, inhibition of Fas signaling or caspase function ablates BRCA1-dependent apoptosis in MCF7.
of Fas-induced apoptosis through caspase and DAXX activation (60, 61), or as an inhibitor of tumor necrosis factor-induced apoptosis, as seen in lymphocytes from JNKK1 and traf2 null animals (62, 63). Our results indicated a different role for JNK. After serum withdrawal, we observed BRCA1-dependent up-regulation of both FasL and Fas. The ability of dn-JNK to abrogate FasL and Fas induction placed JNK upstream of FasL and Fas in the apoptotic signaling pathway. These results are consistent with recent reports implicating JNK-dependent activation of the Fas/FasL pathway in nerve growth factor withdrawal-induced apoptosis in neuronal PC12 cells (33), stress-induced apoptosis in Jurkat cells (35), and cell detachment-associated apoptosis (anoikis) in various cell types (55, 56). These results also extend these previous studies by demonstrating a role for BRCA1 in modulating signaling through the JNK/Fas/Fas pathway.

The ability of the blocking antibodies ZB4 and Nok2 to abrogate BRCA1-dependent serum withdrawal-induced apoptosis provided strong evidence that the up-regulation of FasL and its interaction with Fas are critical to this death process. Consistent with these results, abrogation of FADD signaling and inhibition of caspase-8 (through expression of CrmA) also inhibited the apoptotic response to serum withdrawal. In addition, expression of dn-caspase-9 inhibited this apoptotic pathway, suggesting that activation of caspase-8 results in activation of caspase-9 through a mitochondrial pathway, as has been suggested for “type II” cells (37, 39, 64). Furthermore, because this apoptotic pathway appears to retain activity in the absence of caspase-3, which is known to be down-regulated in MCF7 cells (37), the suggestion is that the caspase-3 effector is not required for this effect. Thus, it is likely that other effector caspases, such as caspase-7, may also mediate processing of multiple cellular targets as part of the BRCA1-dependent apoptotic pathway.

A number of studies have reported that FasL/Fas signaling after DNA damage requires the action of the tumor suppressor protein p53 (42–44). In the present study, we determined whether loss of p53 affected the BRCA1-dependent serum withdrawal-induced FasL/Fas pathway. The signal transduction pathway defined above was activated in a BRCA1-dependent manner in two cell models lacking p53 function. These results suggest that activation of the Fas/FasL pathway by DNA damage withdrawal proceeds by a pathway that is distinct from DNA damage-induced Fas/FasL activation.

In summary, we have established that the BRCA1 tumor suppressor function as a regulator of apoptosis in response to serum deprivation and a number of other apoptotic stimuli. We have delineated a BRCA1-dependent, serum withdrawal-induced apoptotic pathway that sequentially involves H-Ras, MEKK4, and JNK followed by induction of FasL and activation of FADD- and caspase-9-dependent signaling. In addition, we have demonstrated that activation of this pathway is p53-independent and we have identified novel dominant negative activity of BRCA1 truncation mutants. These results provide an improved understanding of the tumor suppressor function of BRCA1 and suggest that the multifunctional BRCA1 protein coordinately regulates apoptotic events in addition to its effects on the DNA damage response, cell cycle progression, and transcription.
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