A Functional Comparison of BRCA1 C-terminal Domains in Transcription Activation and Chromatin Remodeling*

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The BRCA1 C-terminal (BRCT) domain is present in a number of proteins that are involved in various aspects of chromosomal events. The BRCT domain of BRCA1 is important for its function in DNA repair and transcriptional activation. When tethered to chromosomal DNA, this region of BRCA1 is capable of inducing changes in chromatin structure. Despite the sequence homology and functional proximity shared by the BRCT-containing proteins, it is not clear whether different BRCT domains confer a common biochemical activity. Much less is known about the functional significance of the characteristic amino acid residues in the BRCT motif. Here, we show that chromatin remodeling and transcription activation is not a common feature of BRCT domains. However, the BRCT domain of the multi-functional repressor-activator protein 1 (RAP1) can activate transcription and remodel chromatin in a manner similar to that shown for the BRCA1 BRCT domain. Most of the conserved amino acid residues in the second BRCA1 BRCT domain are essential for its function in transcriptional activation. In contrast, mutations of many analogous amino acid residues in the RAP1 BRCT domain greatly elevate the transcriptional activity. These data indicate that the conserved residues in these two BRCT domains may play different roles in transcriptional activation.

Mutations of BRCA1 account for a large proportion of familial breast and ovarian cancers (1, 2). Multiple lines of evidence suggest that BRCA1 is involved in regulation of several nuclear functions, including transcription, DNA repair, recombination, and checkpoint control (3–11). The entire 1863 amino acid protein contains a highly conserved RING finger domain at the N terminus and two repeats of the BRCA1 C-terminal (BRCT)1 domain at the C terminus. Although most cancer-predisposing mutations of BRCA1 result in gross truncation of the protein, 5–10% of the disease-associated mutations lead to single amino acid substitutions, many of which occur in the RING or BRCT domains, suggesting that both domains are pivotal to BRCA1 function in tumor suppression.

The BRCT domain of BRCA1 is required for its function in both transcriptional activation and DNA repair (3–10). When tethered via the GAL4 DNA binding domain (DBD), the BRCT domain is capable of stimulating transcription from a GAL4-responsive reporter gene (3, 4) and remodeling chromatin from chromosome-embedded GAL4 binding sites (13). The same region is also reported to interact with histone-modifying enzymes such as the histone acetyltransferase p300 (14, 15) and the human histone deacetylase, HDAC (16). Importantly, cancer-predisposing mutations in this region abolish the ability of BRCA1 to activate transcription, enhance DNA repair efficiency, and remodel chromatin. Thus, BRCA1 may utilize the BRCT domain to increase chromatin accessibility and facilitate multiple chromosomal events.

The BRCA1 BRCT domain also shares a limited sequence homology with a large number of proteins that are involved in various aspects of chromosomal events, such as DNA repair, replication, recombination, gene activation and silencing, and checkpoint control (17, 18). The functional proximity of the BRCT superfamily opens up the possibility that BRCT domains may share a common activity in regulation of nuclear functions. Several recent reports suggest a role of BRCT domains in mediating protein-protein interactions with each other or with a different structural module. For example, the BRCT domains present in DNA ligase III and XRCC1, two mammalian DNA repair proteins, interact strongly with each other (19). In addition, the BRCT domain of BRCA1 binds CtIP, a transcriptional co-repressor (20–22). Despite these findings, it remains to be determined whether different BRCT domains utilize a common biochemical feature to regulate various nuclear processes.

The BRCT motif is an approximately 100-amino acid long region defined by distinct conserved patches of hydrophobic residues (17, 18) (also see the alignment in Fig. 3). An x-ray crystallographic study of the second BRCT domain of the repair protein XRCC1 shows that it contains a four-stranded parallel β-sheet encircled by three α-helices (23). Based on the XRCC1 BRCT structure, a model for the BRCT domain of BRCA1 has been constructed, and a few cancer-predisposing mutations in this region are predicted to either disrupt the interface or cause incorrect folding (23). However, most of the characteristic amino acid residues in the BRCA1 BRCT domain have not been associated with familial breast or ovarian cancers. Therefore, the relevance of these conserved residues to BRCA1 function remains to be explored.

In this study, we compared the potentials of the BRCT domains from BRCA1 and several yeast proteins in transcription activation and chromatin remodeling. Our work shows that most BRCT domains, with the exception of the scRAP1 BRCT domain, do not display in vivo properties similar to those shown for the BRCA1 BRCT domain. Mutational study of the BRCA1 and scRAP1 BRCT domains also indicates that many of the characteristic amino acid residues of the BRCT motif play
distinct roles in transcriptional activation by these BRCT domains. Taken together, our findings point to the functional diversity and structural complexity among the BRCT superfamily members.

EXPERIMENTAL PROCEDURES

Plasmids and Cells—For the yeast-based transcription assay shown in Fig. 1C, the lacZ reporter plasmid carrying two GAL4 binding sites was integrated into the yeast strain RL1 as described previously (24). To construct the reporter plasmid with five GAL4 binding sites used in Fig. 5B, an XbaI-HindIII fragment containing the five GAL4 sites from the vector G5BCAT (25) was blunt-ended and cloned into the blunt-ended Xhol site in the lacZ reporter construct pJL638 (26). The resulting plasmid was linearized with Stul and integrated at the URA3 locus. The luciferase reporter plasmid used in human cells contains four GAL4 binding sites in front of the fos TATA element as described previously (27).

Site-directed mutagenesis was performed using the Kunkel method as described (28). To construct the yeast expression vectors for the GAL4 fusion proteins, the sequences encoding the following BRCT domains were amplified by a standard polymerase chain reaction method: BRCA1 (aa 1560–1863); DPB11 (Fig. 1A; fragments a and b; aa 1–220); DPB11 (fragments c and d; aa 222–579); ESC4 (fragments e and f; aa 841–1070); RAD9 (aa 1027–1309); RAP1 (aa 121–208); and RFC1 (aa 153–243). The polymerase chain reaction fragments were subsequently cloned into the XbaI and BamHI sites immediately downstream of the HA-GAL4(1–94) sequence in the CUP1 expression vector described previously (24). The entire sequence of the amplified fragments was verified by sequencing. The resulting expression vectors were integrated into the LEU2 locus on the chromosome. For expression of the fusion proteins in human cells, the BRCT domain of BRCA1 was cloned into the XbaI-BamHI sites in the expression vector pCG-GAL4(1–94)-HA (27).

The RL1 yeast strain used for the micrococcal nuclease (MNase) sensitivity assay and the β-galactosidase assay was described previously (27). Human HEK293T cells, generously provided by T. Ouchi at the Mount Sinai School of Medicine, were maintained in Dulbecco’s modified Eagle’s medium with 10% fetal calf serum.

Immunoblotting and MNase Assay—These assays were performed as described previously (27), except that all liquid growth media contained 100 μg/ml copper sulfate for induction of the GAL4 derivatives. The immunoblots were probed with an anti-HA antibody (12C5).

Transcription Assays—The yeast transcription assay was performed and the specific activity calculated following a standard protocol (29). For the luciferase assay, human HEK293T cells were transfected using LipofectAMINE 2000 from Life Technologies, Inc. The following plasmids were included in the transfection: the luciferase reporter (0.5 μg), the specific activity calculated following a standard protocol (29).

RESULTS AND DISCUSSION

Transcription Activation Is Not a Universal Feature of BRCT Domains—To test the possibility that BRCT domains besides that of BRCA1 may also be capable of activating transcription, we fused the hemagglutinin (HA)-tagged GAL4-DBD to the BRCT domains of several proteins from Saccharomyces cerevisiae (Fig. 1A). The BRCT-containing proteins chosen here are known for their roles in the regulation of various aspects of chromosomal events. These proteins include Dpb11p, involved in DNA replication and S-phase checkpoint (30); Rfc1p, in DNA replication and repair (31); Rad9p, in DNA damage checkpoint (32); Rap1p, in transcriptional activation, silencing, and telomere length maintenance (33, 34); and Esc4p, in gene silencing (17). As shown in Fig. 1B, all GAL4 fusion proteins were expressed with the expected sizes and at a comparable level in yeast.

The potential of these fusion proteins to activate transcription in yeast was analyzed using a GAL4-responsive lacZ reporter gene (Fig. 1C). Consistent with previous findings (3, 4), GAL4-BRCA1 activated transcription in yeast as well as human cells (compare columns I and 2 in Fig. 1C; also see Fig. 4).

Of all the other BRCT fusion proteins tested, only GAL4-RAP1 gave rise to an elevated β-galactosidase activity (compare columns I with 7). None of the remaining GAL4-BRCT constructs showed any appreciable levels of transcriptional stimulation (columns 3–6 and 8). Although it remains possible that the lack...
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Most of the Conserved Residues in the BRCA1 BRCT Domain Are Critical for Transcriptional Activation—To address the significance of the characteristic amino acid residues of the BRCT motif in transcriptional activation, we introduced mutations in several conserved blocks of the second BRCT domain of BRCA1 (Hs BRCA1-b in Fig. 3). These include the N-terminal tail, α1, α3, and the C-terminal tail as predicted based on the structure of the XRCC1 BRCT domain (23). The four-stranded β-sheet and α2 were not targeted for mutagenesis in this study, as the β-sheet is predicted to form the core of the structure and the corresponding mutant proteins tend to be unstable (data not shown), whereas the sequence for α2 is not universally present in the BRCT superfamily.

A total of 11 alanine substitution mutants were constructed in the context of the GAL4-BRCA1 fusion. To analyze the mutational effects in a more physiological context, the wild-type and mutant fusion proteins were expressed and their transcriptional activity assessed in human HEK293T cells (Fig. 4, A and B). As expected, the wild-type GAL4-BRCA1 fusion can potently stimulate transcription from a GAL4-responsive luciferase reporter construct (compare lane 2 with lane 1 in Fig. 4B). Mutations at multiple conserved amino acid residues either reduced or completely abolished the transcriptional activity. For example, I1760A/F1761A, L1780A, M1783AV1784A, G1788A, V1838A/L1839A, S1841A, and Y1853A/L1854A/I1855A all severely impaired the trans-activation capability of the BRCT domain (compare lane 2 with lanes 3, 6, 8–11, and 13 in Fig. 4B). Of all the conserved residues mutated in this study, only L1764A failed to affect BRCT function in a significant manner (lane 4 in Fig. 4B). In addition, mutations of three nonconserved residues (D1778A, W1782A, and Q1848A) did not cause deleterious effects either (Fig. 4B, lanes 5, 7, and 12), despite the fact that the analogous residues of Asp1778 and Trp1782 in XRCC1 are involved in dimer formation between two BRCT domains (23). The behaviors of the mutant proteins are summarized below the sequence of the BRCT domain in Fig. 3.

Natural mutations at Met1778, Gly1788, and Ser1841 have been identified as unclassified mutant variants in terms of cancer predisposition and predicted to affect protein folding (23). The alanine substitution mutants at these three positions indeed abrogated the transcriptional activation by GAL4-BRCA1 (lanes 8, 9, and 11 in Fig. 4B), suggesting that the corresponding natural mutations at these sites may represent genuine cancer-predisposing mutations. Taken together, the data strongly suggest that a majority of the conserved amino acid residues in the BRCT domain are critical for BRCA1 function in transcriptional activation. Our results in human cells are also in agreement with a recent yeast-based study of the BRCA1 BRCT domain (35). In particular, the three regions mutagenized in both studies (Phe1761, Leu1780, and Tyr1853-Leu1854-Ile1855) are important for trans-activation in human as well as yeast cells.

Distinct Roles of the Conserved Residues in the scRAP1 BRCT Domain—Given the sequence homology and functional similarity between the BRCA1 and scRAP1 BRCT domains, we asked whether the analogous residues in the scRAP1 BRCT domain are critical for transcriptional activation as well. Site-directed mutagenesis was employed to change the amino acid residues analogous to those in the BRCA1 BRCT domain studied above. As illustrated in Fig. 3, alanine substitutions were introduced at Pro125-Leu126 of the N terminus; Leu143, Asn144, Leu149, Arg151, and Leu152-Ile153 of α1; Gly157-Gly158 between α1 and β2; Tyr190, Ile191-Lys192, and Cys194 of α3; and Tyr206-Leu207-Val208 of the C-terminal tail.

The potentials of the mutant GAL4-RAP1 fusion proteins in
transcription activation were analyzed in a yeast β-galactosidase assay. As shown in Fig. 5B, two mutations in α3 (Y190A and C194A) and one in the C-terminal tail (Y206A/L207A/V208A) either reduced or completely abolished the ability of GAL4-RAP1 to stimulate transcription (Fig. 5B, lanes 5–7). These mutants were expressed at a similar level as the wild-type protein (compare lanes 1, 8, 9, and 12 in Fig. 5A). Notably, the corresponding residues in the BRCA1 BRCT domain had a similar deleterious effect on transcriptional activation (see W1837R in Ref. 13 and S1841A and Y1853A/L1854A/I1855A in Fig. 4B). Furthermore, both W1837R and a nonsense mutation that results in deletion of Tyr1853-Leu1854-Ile1855 in BRCA1 are found to be associated with familial breast cancer. Therefore, the conserved residues toward the C terminus of the BRCT motif are required for both BRCA1 and scRAP1 BRCT domains to stimulate transcription.

In contrast to the three mutations mentioned above, changes at many other conserved positions in the scRAP1 BRCT domain greatly increased the transcriptional activity of the fusion protein (compare lane 8 with lanes 9–13 in Fig. 5B; also note the two scales for lanes 1–7 and 8–13). Most strikingly, P125A/L126A, L152A/I153A, and G157A/G158A were at least 15-fold as robust as the wild-type protein (compare lane 8 with lanes 9, 11, and 12 in Fig. 5B). In addition, L149A and I191A/K192A also resulted in a significant increase in β-galactosidase activity, albeit to a lesser extent (lanes 10 and 13). Interestingly, some of these “superactivating” mutants were expressed at lower levels than the wild-type GAL4-RAP1 (lanes 4, 6, 7, and 10 in Fig. 5A). Therefore, as summarized in Fig. 3, although the
C termini of the BRCA1 and scRAP1 BRCT domains are required for both domains to activate transcription, many of the characteristic amino acid residues of the BRCT motif appear to play distinct roles in these two BRCT domains. This finding further supports the notion that the signature residues of the BRCT motif are not sufficient to confer a common function among the BRCT superfamily members.

Based on the crystal structure of the XRCC1 BRCT domain (23), the residues involved in repressing the BRCT function in scRAP1 (Pro125-Leu126, Leu149, Leu152-Ile153, Gly157-Gly158, and Ile191-Lys192) are predicted to reside proximally in the tertiary structure. Moreover, Leu149 and Leu152-Ile153 of α1 and Ile191-Lys192 of α3 are likely to be involved in mediating the intramolecular interaction between α1 and α3, whereas the highly conserved Gly-Gly residues between α1 and β2 may be important for proper orientation of the two helices. Thus, it is conceivable that the interaction between α1 and α3 may result in a conformation that is unfavorable for transcriptional activation by the RAP1 BRCT domain. Alternatively, the amino acid residues of interest may be involved in an interaction with a transcriptional repressor. In either situation, the negative regulatory region of the scRAP1 BRCT domain may serve as a molecular switch that coordinates the multiple functions of RAP1 in transcriptional activation, gene silencing (33), and telomere length maintenance (36), etc. However, it remains formally possible that the scRAP1 BRCT domain may fold in a conformation distinct from that of the BRCA1 and XRCC1 BRCT domains.

The scRAP1 BRCT domain is identified by sequence comparison, yet its role in the biological function of the full-length protein remains obscure. In fact, the entire N-terminal sequence including the BRCT domain is not essential for scRAP1 to support cell viability (37). In this regard, it is somewhat puzzling that the BRCT domain is well conserved between the yeast and human RAP1 proteins, whereas the sequence for the potential trans-activation domain at the C terminus of scRAP1 is not present as such in hRAP1 (38). Although the exact contribution of the BRCT domain to RAP1 function awaits further investigation, it is possible that the transcriptional activation and chromatin remodeling property associated with the BRCT domain may be important for a more specialized, nonessential function of scRAP1. It is also tempting to speculate that, in the absence of a strong trans-activation domain in hRAP1, the BRCT domain may play a more prominent role in RAP1-mediated regulation of chromosomal events in human cells.

In conclusion, the current work demonstrates that most of the characteristic amino acid residues of the BRCA1 BRCT domain are required for its function in transcription activation. However, these conserved residues are not sufficient to confer a similar in vivo activity on other members of the BRCT superfamily. The study also shows that the evolutionarily conserved BRCT domain in RAP1 is capable of transcription activation and chromatin remodeling. However, unlike the BRCA1 BRCT domain, many of the characteristic residues in the RAP1 BRCT domain are involved in negative regulation of transcription activation. Thus, there appears to be a significant degree of structural complexity and functional diversity among different BRCT domains.

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