BRCA1 can stimulate gene transcription by a unique mechanism

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Most familial breast and ovarian cancers have been linked to mutations in the BRCA1 gene. BRCA1 has been shown to affect gene transcription but how it does so remains elusive. Here we show that BRCA1 can stimulate transcription without the requirement for a DNA-tethering function in mammalian and yeast cells. Furthermore, the BRCA1 C-terminal region can stimulate transcription of the p53-responsive promoter, MDM2. Unlike many enhancer-specific activators, non-tethered BRCA1 does not require a functional TATA element to stimulate transcription. Our results suggest that BRCA1 can enhance transcription by a function additional to recruiting the transcriptional machinery to a targeted gene.

RESULTS

BRCA1 stimulates transcription without requiring a specific DBD in human cells

INTRODUCTION

BRCA1 mutations are thought to account for ~45% of families with high breast cancer risk and >80% of families with high risk of early-onset breast and ovarian cancer (Easton et al., 1993). The human BRCA1 gene encodes a 1863 amino acid nuclear protein that has been implicated in DNA repair and transcription activation (see Monteiro, 2000; Welch et al., 2000). A role for BRCA1 in gene transcription has been proposed mainly for the following reasons: (i) the C-terminal portion of BRCA1, which bears an excess of negatively charged residues, can activate transcription of a target gene when attached to a DNA-binding domain (DBD) in vivo (Chapman and Verma, 1996; Monteiro et al., 1996; Anderson et al., 1998) and in vitro using a highly purified system (Haile and Parvin, 1999); (ii) it associates with a form of RNA polymerase II holoenzyme (Scully et al., 1997); and (iii) it modulates the activity of certain transcriptional activators (Somasundaram et al., 1997; Ouchi et al., 1998, 2000; Wang et al., 1998). It is noteworthy that the above mentioned work implies BRCA1 as a physiological regulator of p53-dependent genes such as p21WAF and MDM2.

Here we show that BRCA1 can stimulate gene transcription of a variety of reporter gene constructs without the requirement for a specific DBD in human and in yeast cells both in vivo and in vitro. We further show that the BRCA1 C-terminal region can increase transcription of a reporter gene independently of a functional TATA element.

Most familial breast and ovarian cancers have been linked to mutations in the BRCA1 gene. BRCA1 has been shown to affect gene transcription but how it does so remains elusive. Here we show that BRCA1 can stimulate transcription without the requirement for a DNA-tethering function in mammalian and yeast cells. Furthermore, the BRCA1 C-terminal region can stimulate transcription of the p53-responsive promoter, MDM2. Unlike many enhancer-specific activators, non-tethered BRCA1 does not require a functional TATA element to stimulate transcription. Our results suggest that BRCA1 can enhance transcription by a function additional to recruiting the transcriptional machinery to a targeted gene.
BRCA1 stimulates transcription by a unique mechanism

Next, we tested whether the BRCA1 C-terminal fragment could stimulate transcription in vitro without the requirement for a DBD. Using a HeLa nuclear extract, we tested the activation potential of recombinant BRCA1(1528–1863) in vitro using a DNA template bearing the AdML core promoter. We found that the recombinant BRCA1 fragment could stimulate transcription up to 7-fold (Figure 1C), a result consistent with those of our transient transfection experiments. A similar BRCA1 fragment bearing the cancer-associated Y1853X mutation, which results in deletion of the last 11 amino acids (Friedman et al., 1994), had no significant stimulatory effect on transcription (Figure 1C, right panel, lane 3).

Transcription stimulation of MDM2 by BRCA1 derivatives

Previous reports have suggested that p53-responsive genes were targets of BRCA1-mediated transcription enhancement. Moreover, p53 has been shown to interact physically with residues 224–500 of BRCA1 (Ouchi et al., 1998; Zhang et al., 1998). Although physical interaction between these two molecules surely contributes in recruiting BRCA1 to p53-responsive genes, it is conceivable that proper overexpression of the BRCA1 activation-effector region would also result in stimulation of the target gene. We thus wanted to test whether the BRCA1 C-terminal region was sufficient, as compared with the full-length molecule, to stimulate transcription of an MDM2-luciferase reporter template (Ouchi et al., 1998). To test this, we transfected HCC1937 cells with vectors expressing C-terminal and full-length BRCA1 derivatives (wild-type and Y1853X mutants), and luciferase activity was analyzed. The results shown in Figure 2 demonstrate that the C-terminal BRCA1 fragment, as well as full-length BRCA1, can efficiently induce transcription elicited at the MDM2 promoter while the Y1853X mutants did not stimulate the reporter as efficiently. These results suggest that overexpression of BRCA1 can bypass the requirement for a p53–BRCA1 interaction in order to stimulate a responsive gene.

The BRCA1 C-terminal region stimulates transcription of a TATA-mutated promoter

In an effort to discriminate further the mechanism of action of BRCA1, as compared with enhancer-binding activators, we set out to test whether BRCA1 required a functional TATA element to stimulate transcription. Hence, we determined the ability of BRCA1 to activate transcription at a reporter template that has a mutation in the TATA element (TGTA) (Figure 3A). This mutation prevents or severely reduces TBP binding to the core promoter, nearly abolishing the ability of a classical activator to activate transcription in certain contexts (Bryant et al., 1996). Thus, the reporter templates used in this experiment have either a wild-type c-fos TATA or a mutated TGTA element both with Gal4-binding sites upstream and the luciferase reporter gene downstream of the transcription initiation site (Bryant et al., 1996). While the acidic activators Gal4–VP16 and Gal4–E2F1 are able to activate transcription efficiently at the TATA template, both activators are severely crippled in their ability to stimulate transcription at the TGTA template. Surprisingly, BRCA1(1528–1863) was able to stimulate transcription even more efficiently at

whether the effect could be specific to the promoter context of the template, we made use of a promoter template bearing two LexA-binding sites in place of the Gal4 sites, and the E1B core promoter in place of the c-fos promoter. The results show that even though both the Gal4–BRCA1 fusion and BRCA1 are not expected to bind the promoter template, they are both able to stimulate transcription when overexpressed in the cell (Figure 1B). These results would be expected for an activator that does not require DNA binding to exert its function.

Fig. 1. BRCA1 does not require specific DNA binding to enhance gene transcription. (A) Activation by Gal4–BRCA1(1528–1863) and BRCA1(1528–1863) at a reporter template bearing Gal4 sites in HCC1937 cells. Transfected cells were assayed for luciferase activity using a reporter template (0.1 μg) bearing four Gal4-binding sites upstream of the c-fos TATA element. Increasing amounts (up to 1.0 μg DNA) of either Gal4–BRCA1(1528–1863) or BRCA1-C(1528–1863) constructs were co-transfected along with the reporter. Activation for BRCA1 and Gal4–BRCA1 is represented as fold increase over activity obtained with the pcDNA3 vector and the Gal4 DBD, respectively. (B) Activation by Gal4–BRCA1 and BRCA1-C at a reporter template lacking Gal4 sites. Transfected HCC1937 cells were assayed for luciferase activity using 0.1 μg of a reporter bearing two LexA sites upstream of the E1B TATA element. The BRCA1 plasmid constructs were transiently transfected as in (A) and activation is represented as the fold increase over activity obtained with the pcDNA3 vector for both constructs. (C) BRCA1 can stimulate gene transcription in vitro. In vitro transcription reactions were carried out with a HeLa nuclear extract and an AdML template. Primer extensions were carried out to measure the extent of activation. Recombinant BRCA1(1528–1863) was added at 100 and 400 ng (left panel, lanes 2 and 3, respectively). The right panel shows that a recombinant Y1853X-bearing mutant (200 ng, lane 3) does not significantly stimulate transcription as compared with the wild-type protein fragment (200 ng, lane 2).
the TGTA template as compared with the TATA template (Figure 3A, right panel). Introduction of a Y1853X mutation markedly impaired stimulation of transcription.

We next wanted to test whether the BRCA1 C-terminal region could further enhance transcription of a gene activated by artificially recruited TBP. Figure 3B shows that TBP, when fused to the Gal4 DBD, can activate transcription some 15-fold in human HEK-293 cells. When BRCA1(1528–1863) is transfected along with Gal4–TBP, we see a \( \sim 3 \) -fold increase in activation by Gal4–TBP. Transfection of the Y1853X BRCA1 mutant did not enhance transcription of Gal4–TBP nearly as efficiently as its wild-type counterpart. These results suggest that BRCA1 can stimulate transcription above levels elicited by artificial recruitment of TBP to the TATA box. We cannot, however, exclude the possibility that BRCA1 further enhances TBP binding to the promoter beyond the recruiting effect of the Gal4 DBD.

**BRCA1 stimulates transcription without requiring a specific DBD in yeast cells**

To determine whether the effect of BRCA1 on transcription was limited to mammalian cells, we performed experiments in yeast. Figure 4A shows that, when expressed in yeast cells, full-length BRCA1 is able to stimulate transcription (bar 2) of a GAL1::lacZ reporter template efficiently. Expression of the Y1853X mutant (Figure 4A, bar 4) in yeast failed to activate transcription, while the C61G mutant (bar 3), also a clinically relevant mutation but located at the N-terminus of BRCA1 (Friedman et al., 1994), could elicit levels of activation comparable to the wild-type protein. The N-terminal region of BRCA1 seems dispensable for this function, confirming our data from mammalian cells where the C-terminal alone is sufficient for activation. Figure 4B shows that recombinant BRCA1(1528–1863) can stimulate transcription of the GAL1 promoter in vitro using a yeast nuclear extract preparation (Wu et al., 1996). The figure also shows that, as in Figure 1C, BRCA1 can stimulate transcription in a dose-dependent fashion. The Y1853X mutant had no stimulatory effect (Figure 4B, right panel, lane 3).
Our results show that the BRCA1 C-terminal region has the surprising ability to stimulate transcription without the need for a DNA-binding function and, in so doing, exploits a mechanism different from one typified by many enhancer-binding activators. Importantly, these activities were crippled upon introduction of a cancer-associated mutation, Y1853X, which destabilizes the BRCT region leaving one of the two BRCT motifs intact. This intact BRCT could perhaps explain the transcriptional activity of that mutant when it is well overexpressed in some experiments. We have also shown that the C-terminal region of BRCA1, as well as the full-length molecule, could stimulate transcription of a p53-responsive promoter. The experiments described here rely on overexpression of BRCA1 derivatives and could imply a global effect on gene transcription, considering that BRCA1 can activate genes with a variety of promoter contexts. However, we propose that our experimental conditions represent increased local concentrations of BRCA1 under physiological conditions, a condition that we believe bypasses specific targeting of BRCA1 to our reporters, a situation well exemplified by our experiment with the MDM2 reporter.

Our results showing that BRCA1 does not require a functional TATA element to stimulate transcription could imply, for example, that either BRCA1 stabilizes binding of the transcription machinery (e.g. TFIIID) to the mutated promoter or, alternatively, BRCA1 could act independently of TBP binding to DNA to stimulate transcription. Recently, oligonucleotide array-based expression profiling experiments have revealed that BRCA1 expression could efficiently induce transcription of the DNA damage-responsive gene GADD45 in a p53-independent fashion (Harkin et al., 1999). Interestingly, examination of the GADD45 proximal promoter region did not reveal the presence of any consensus TATA element (not shown). Thus, the fact that BRCA1 does not require a functional TATA element to stimulate transcription would be relevant in this particular case.

In prokaryotes, there are at least three examples of activators that do not require sequence-specific DNA-binding activity in order to stimulate transcription: (i) DNA-tracking proteins exemplified by the phage T4 Gp54 one-dimensional sliding clamp; (ii) the phage N4 single-stranded binding protein (N4SSB), which interacts with the β′ subunit of RNA polymerase and activates σ70-type promoters; and (iii) σ54-specific activators, which, even if being enhancer-binding proteins, can activate transcription at high concentrations without being DNA tethered (see Ptashne and Gann, 1997; Hochschild and Dove, 1998). It is conceivable that BRCA1 could stimulate transcription by a mechanism similar to any of the bacterial examples listed above.

Recent experiments carried out with the TATA-binding protein-interacting protein TIP120 and ABT1 have shown that they too can enhance transcription without requiring a DNA-tethering function (Makino et al., 1999; Oda et al., 2000). Interestingly, our results could explain why BRCA1 activates the p21WAF1 gene independently of p53 (Somasundaram et al., 1997; Ouchi et al., 1998) and also IFN-γ target genes in the absence of interferon stimulation (Ouchi et al., 2000).

We consider that the mechanism by which BRCA1 stimulates transcription could constitute an advantage in cases where the transcriptional machinery might be paused, for example, at sites of DNA damage in a particular gene, located at a distance from its enhancer sequences. In the latter case, BRCA1 would be able to counteract a transcriptional pause without any interaction(s) with the upstream enhancer. Consistent with this idea is a recent report which suggests that BRCA1 BRCT domains can bind DNA.

**DISCUSSION**

Our results show that the BRCA1 C-terminal region has the surprising ability to stimulate transcription without the need for...
strand breaks and termini (Yamane and Tsuruo, 1999). Thus, it is conceivable that BRCA1 could recognize damaged DNA at paused transcription sites, and in so doing, increase favorable interactions with the transcriptional machinery thereby further enhancing transcription and perhaps, the transcription-coupled DNA repair process itself. In an alternative mode not mutually exclusive with the previous one, BRCA1 could be targeted to specific genes by interaction with enhancer-binding activators and in so doing, further enhance the transcription process.

METHODS

Transient transfections. Human HCC1937 and HEK-293 cells were used for the transfection experiments and maintained in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal calf serum. Cells were plated in six-well tissue culture plates for 24 h before transfection at a density of 1–2 × 10^5 cells/well. Cells were then transfected using either Fugene 6 or Lipofectamine 2000 reagents. After 18–24 h, cells were processed for luciferase assays. Transfection efficiencies, when appropriate, were normalized using the pGL3 luciferase vector; in some experiments, we noticed that BRCA1 increased the activity of our lacZ internal control, thus, in many experiments we did not normalize our transfection efficiencies; nonetheless we always measured lacZ values to make sure no gross deviations would be observed. Details of plasmid constructions are available upon request.

Recombinant BRCA1 derivatives. Recombinant BRCA1 (1528–1863) was expressed in *Escherichia coli* using the pET30a expression vector. The recombinant protein was first chromatographed on Ni-NTA agarose and then further fractionated on an FPLC mono-Q ion exchange column. The Y1B3X mutant fragment was also expressed in *E. coli*, affinity purified on Ni-NTA agarose and then subjected to chromatography on a Superdex 200 HR 10/30 column.

Nuclear extracts and in vitro transcription. HeLa and HCC1937 nuclear extract preparation and transcription reactions were as previously described (Shapiro et al., 1988). Yeast nuclear extract preparation and transcription reactions were as described in Wu et al. (1996) and supplemented with recombinant TBP and TFIIIE. For human in vitro transcription experiments, the pGML4 template was used at 100 ng/reaction; pGML4 bears the AdML promoter without any enhancer sequences. For yeast in vitro transcription experiments, the pGDC01 template was used at 100 ng/reaction; pGDC01 bears the GAL1 promoter with its native UASG. All data were quantified by phosphorimaging.

Yeast manipulations. The yeast strain TGY14 (MATa, ura3-251-373-328, leu2, pep4.3) was used and contained a GAL1::lacZ reporter gene inserted downstream of an ERE. Competent yeast cells were obtained using the yeast transformation system (Clontech) based on lithium acetate, and cells were transformed according to the manufacturer’s instructions. Activity of the reporter was measured by liquid β-galactosidase assays.

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