

Profiling of the BRCA1 transcriptome through microarray and ChIP-chip analysis

Julia J. Gorski^{1,*}, Kienan I. Savage¹, Jude M. Mulligan², Simon S. McDade¹,
Jaine K. Blayney¹, Zhaoping Ge² and D. Paul Harkin^{1,2}

¹Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast BT9 7BL and
²ALMAC Diagnostics, Craigavon BT63 5QD, UK

Received March 2, 2011; Revised July 27, 2011; Accepted August 1, 2011

ABSTRACT

A role for BRCA1 in the direct and indirect regulation of transcription is well established. However, a comprehensive view of the degree to which BRCA1 impacts transcriptional regulation on a genome-wide level has not been defined. We performed genome-wide expression profiling and ChIP-chip analysis, comparison of which revealed that although BRCA1 depletion results in transcriptional changes in 1294 genes, only 44 of these are promoter bound by BRCA1. However, 27% of these transcripts were linked to transcriptional regulation possibly explaining the large number of indirect transcriptional changes observed by microarray analysis. We show that no specific consensus sequence exists for BRCA1 DNA binding but rather demonstrate the presence of a number of known and novel transcription factor (TF)- binding sites commonly found on BRCA1 bound promoters. Co-immunoprecipitations confirmed that BRCA1 interacts with a number of these TFs including AP2- α , PAX2 and ZF5. Finally, we show that BRCA1 is bound to a subset of promoters of genes that are not altered by BRCA1 loss, but are transcriptionally regulated in a BRCA1-dependent manner upon DNA damage. These data suggest a model, whereby BRCA1 is present on defined promoters as part of an inactive complex poised to respond to various genotoxic stimuli.

INTRODUCTION

One of the major functions of BRCA1 is its role in transcription, first proposed upon the discovery of highly conserved regions of acidic amino acids in its C-terminus, and later with reporter assays using BRCA1–GAL4 DNA fusions (1). A physiological role for BRCA1 transcriptional activity was suggested by the finding that pathogenic

mutations within the BRCA1 C-terminus abolished GAL4–BRCA1-mediated transactivation and growth suppression (1,2). Furthermore, BRCA1 co-purifies in a complex with the RNA Polymerase II holoenzyme via interaction with RNA helicase A, and transcriptional activation by this complex was found to require BRCA1 (3,4). BRCA1 binds DNA directly, prompting the idea that BRCA1 binds to genetic promoters and, through interaction with the core transcriptional machinery, directly affects transcription (1). However, more recent evidence suggests that BRCA1 does not bind to DNA in a sequence-specific manner and that its direct DNA binding activity may be restricted to branched DNA structures, consistent with its role in DNA repair (5).

Extensive research has revealed that BRCA1 regulates transcription in a number of ways. BRCA1 can regulate signalling pathways that affect transcription factor (TF) activation. For example, BRCA1 phosphorylation in response to DNA damage is required for ATM (Ataxia Telangiectasia Mutated)-mediated p53 phosphorylation and activation, regulating the p53-dependent G1/S checkpoint (6). Furthermore, BRCA1 binds to ER α and represses both ligand-dependent and independent ER α signalling, affecting E2/ER α -dependent transcription (7,8).

In addition to this 'indirect' role in transcriptional regulation, BRCA1 has been identified on a number of target gene promoters where it directly influences gene expression (9–13). Given BRCA1's lack of sequence-specific DNA binding, BRCA1 is likely to be recruited to promoters by sequence-specific DNA binding TFs. Consistent with this, BRCA1 interacts with a large pool of TFs, many of which recruit BRCA1 to promoters (14,15). When recruited to promoters, BRCA1 may act as either a transcriptional co-activator or co-repressor, dependent upon the TF(s) and other accessory factors, including chromatin remodelling factors, bound at the specific promoter.

BRCA1 binds to a number of chromatin remodelling factors such as HDAC1 and HDAC2, and with the BRG1 and BRD7 subunits of the SWI/SNF complex (13,16,17).

*To whom correspondence should be addressed. Tel: +44 2890972795; Fax: +44 2890263744; Email: j.gorski@qub.ac.uk

BRCA1 also interacts with hGCN5 and TRRAP, forming part of a HAT (Histone Acetyl Transferase) complex which requires BRCA1 for activation (18). Taken together, this suggests that BRCA1 plays an accessory role in transcriptional regulation, modulating the recruitment and activity of various proteins within promoter bound complexes.

Here, we have used a combination of ChIP-chip and microarray-based expression profiling to provide a genome-wide overview of the role played by BRCA1 in transcriptional regulation. We provide evidence to suggest that BRCA1 is recruited to defined promoters through interactions with a range of known and novel TFs. Finally, we suggest that BRCA1's role in these pre-assembled complexes appears to be important for mediating transcriptional responses to defined stimuli, such as ionizing radiation or etoposide treatment.

MATERIALS AND METHODS

Cell lines

MCF7 cells were obtained from the ECACC, Wiltshire, UK, and routinely maintained as detailed (19).

siRNA transfection

Scrambled control siRNA were obtained from Invitrogen. BRCA1 siRNAs were obtained from Qiagen; BRCA1 #2: 5'CAGGAAATGGCTGAACTAGAA 3', BRCA1 #3: 5'ACCATACAGCTTCATAAATAA 3'. Oligos were delivered to a final concentration of 10 nM by reverse transfection using RNAiMax (Invitrogen, Paisley, UK) according to manufacturer's instructions.

qPCR analysis

A quantity of 2 µg of RNA was reverse transcribed using MMLV (Invitrogen) according to the manufacturer's instructions. The qRT-PCR was performed using primers specific to each transcript, or to actin mRNA. All qRT-PCR primers were designed using the Roche Universal Probe library (www.roche.com) and obtained from MWG (S10).

Clonogenic assays

About 1×10^3 MCF-7 cells were seeded into 6-well plates. After 24 h, the cells were exposed to a medium containing increasing doses of PARP-1 inhibitor (KU0058948). Cell medium was replenished every 4 days. After 12 days, the cells were fixed, stained with crystal violet and counted.

Preparation of nuclear extracts and immunoprecipitations

Co-immunoprecipitations of BRCA1 were carried out from nuclear extracts of 293T HEK cells as described previously (19). A quantity of 2 µg of the monoclonal antibody Ab-1 (Calbiochem), IgG1 (DAKO, Denmark) or HA antibody (Santa Cruz) were used.

Western blotting

Nuclear extracts were prepared and quantitated as described previously (19). Samples were resolved by SDS-PAGE and western blotted for BRCA1 (D9); E2F-1 (sc251); AP2-α (sc-81182); Pax 2 (sc133889); Pax 4 (sc-27832) (Santa-Cruz Biotechnology); PPM1D (WP-401) Bioworld; PDE4DIP (H00009659) stratech; ZF5 (sab1402396) Millipore; NME1 (5353S) NEB.

Gene expression profiling and analysis

RNA labelling and hybridization and analysis was as detailed in previous study (19).

Chromatin immunoprecipitations

ChIP assays were performed as previously described (19). Antibodies used for immunoprecipitation were RNA Polymerase II (CTD4H8, Millipore), BRCA1 (Ab-1; Calbiochem), HA (sc-805, Santa Cruz) rabbit IgG and IgG1 (DAKO). Immunoprecipitated DNA was purified using QIAquick PCR purification kit (Qiagen). Semi-quantitative and qPCR amplification was performed on the purified DNA using primers that were designed to specific regions of BRCA1 bound peaks identified from array analysis (S10). For ChIPs analysed by qPCR, fold enrichment was calculated by first normalizing to input qPCR and then normalizing to the same normalized value calculated for the same ChIP using primers specific to a control region ~2 kp upstream of the S100A7 TSS.

ChIP-chip and analysis

Purified, immunoprecipitated and total DNA were amplified using LMPCR as directed by NimbleGen protocols (see <http://NimbleGen.com> for details). A quantity of 4 µg of immunoprecipitated and total DNA was shipped to NimbleGen Systems for labelling and array hybridization on NimbleGen Human ChIP-chip 385 K refseq Promoter array. This array covers 24 659 transcripts (2200 bp upstream tiling, 500 bp downstream tiling, probes are spaced ~100 bp apart). Analysis and peak identification was performed by Roche NimbleGen. Binding peaks are detected by searching for 4 or more probes whose signals are above the specified cutoff values, ranging from 90% to 15%, using a 500 bp sliding window. The cut-off values are a percentage of a hypothetical maximum, which is the mean + 6 SD. The ratio data are then randomized 20 times to evaluate the probability of 'false positives'. Each peak is then assigned a false discovery rate (FDR) score based on the number of peaks still exceeding the cut-off values after 20 randomizations. Binding peaks with an FDR ≤ 0.2 are considered strong/true binding regions.

RESULTS

Identification of potential BRCA1 regulated genes by microarray expression profiling

To identify transcriptional targets of BRCA1, we performed microarray expression analysis of BRCA1-depleted MCF7 cells using a breast tissue enriched expression array (BrDSA, ALMAC diagnostics). BRCA1 was

depleted in four independent replicate experiments using two different BRCA1-targeted siRNA oligos (BRCA1 II and III, respectively). Control cells were transfected with a scrambled siRNA oligo. BRCA1 depletion was confirmed at both the protein and mRNA level prior to array hybridization (Figure 1A and B). Loss of BRCA1 confers sensitivity to Poly-ADP Ribose Polymerase (PARP) inhibition due to accumulation of unrepaired DNA damage (20,21). Therefore, we confirmed the functionality of BRCA1 depletion for both siRNA oligonucleotides by testing the sensitivity of BRCA1-depleted cells to the PARP inhibitor KU0058948 (20). Cells treated with either BRCA1 siRNA oligo were sensitive to PARP inhibition (IC_{50} 3.126×10^{-9} M and 2.517×10^{-9} M compared with $IC_{50} > 1 \times 10^{-4}$ M for scrambled control) confirming functionality of BRCA1 depletion in these cells (Figure 1C).

BRCA1 differentially expressed gene lists for each siRNA oligo were generated by comparing either BRCA1 II with control or BRCA1 III with control, using all 4 samples in each group. Expression fold change was computed for each probe in each group and both the fold change and *P*-value were used for filtering in order to generate differentially expressed gene lists (fold change ≥ 1.7 , $P \leq 0.05$) (22). Depletion of BRCA1 with either BRCA1 siRNA oligo resulted in altered expression of $\sim 10\%$ of probe sets on the BrDSA relative to control. In order to gain a more precise list of differentially expressed genes following BRCA1 loss, we overlapped the two probe set lists generated (siRNA II and III which contained 5782 and 4630 BRCA1 regulated probe sets, respectively) to produce a final common list containing 2211 probe sets representing 1294 BRCA1-regulated genes (Figure 1D). Heat map analysis of these probe sets shows a clear correlation with BRCA1 loss between the two oligo sets (Figure 1E). Gene Ontology analysis revealed that loss of BRCA1 leads to transcriptional regulation of genes involved in multiple cellular processes previously linked to BRCA1 such as cell cycle arrest, signal transduction, chromatin remodelling and transcription (Supplementary Figure S1).

Identification of direct BRCA1 transcriptional targets by ChIP-chip analysis

It is evident from expression profiling that loss of BRCA1 results in radical changes in transcription profiles. However, it is likely that many of the transcriptional changes are secondary effects mediated by BRCA1-dependent regulation of additional transcriptional regulators, such as ER α (23) and/or as a cellular response to increased genomic instability/cellular stress caused by BRCA1 loss. Therefore, in order to identify direct transcriptional targets of BRCA1, we performed ChIP-chip, using the well-characterized NimbleGen HG18 promoter array. Three independent BRCA1 ChIP assays were performed from MCF7 cells. Prior to hybridization to promoter arrays, we confirmed efficient immunoprecipitation of BRCA1 by western blot analysis (Figure 2A). Specificity of the BRCA1 ChIP was analysed by PCR using primers specific for *S100A7* and *ESR1* promoters, which are known to be directly bound and regulated by

BRCA1 (9,10) (Figure 2B). We also performed PCR using primers upstream of *S100A7* and *ESR1* transcription start sites (TSS) to ensure that the BRCA1 antibody was not precipitating DNA non-specifically (Figure 2B). ChIP-chip data was analysed using NimbleScan. Perl script was generated in order to identify the overlap of binding sites between at least two biological replicates with an enrichment score > 1 and FDR < 0.2 . Using this approach, we identified 980 BRCA1 bound peaks (Supplementary Figure S2). BRCA1 binding peaks from ChIP-chip data were visualized using Integrative Genomic Viewer (IGV) (Broad Institute, MIT), examples of peaks observed for *S100A7* and *ESR1* are shown (Figure 2C and Supplementary Figure S3).

We initially analysed the genomic distribution of BRCA1 bound peaks and determined that BRCA1 binds uniformly to all chromosomes within the genome (Figure 2D). Uniform binding of BRCA1 was confirmed using linear regression and correlation analysis, which revealed no statistically significant BRCA1 binding clusters ($P = 0.790$), indicating that BRCA1 is a genome-wide TF. Given that BRCA1 binds genetic promoters, we performed a search for conserved regions/sequences within all 980 BRCA1 bound peaks using DeNovo sequence analysis software (24,25). We could not identify any conserved DNA sequences common to the BRCA1 bound regions, suggesting that BRCA1 is binding to different genetic promoters through interaction with a range of DNA binding TFs. *Cis*-regulatory Element Annotation System (CEAS <http://liulab.dfci.harvard.edu/CEAS/>) analysis confirmed the majority of BRCA1 bound peaks to be within the proximal promoter region (-1000 bp) of genes (26) (Figure 2E and F), suggesting that BRCA1 is playing an important role in the direct transcriptional regulation of the genes identified. Additionally, CEAS analysis identified a number of binding sites of known BRCA1 interacting TFs such as Sp1, ATF and CREB (Supplementary Figure S4) (27,28).

Since BRCA1 was identified as bound primarily to proximal promoter regions ($\sim 53\%$ of binding sites) and given the difficulty of accurately distinguishing unconfirmed enhancer regions, we performed a consensus TF-binding motif search to BRCA1 bound peaks found within 500 bp of known TSSs. This identified a number of potential BRCA1 recruiting TFs including some previously described BRCA1 interacting/recruiting TFs and the well characterized BRCA1 interacting c-Myc/Max complex (29,30) (Figure 2G).

Comparison of Chip-chip and microarray datasets

In order to identify genes that were differentially expressed upon BRCA1 loss and demonstrated BRCA1 promoter binding, we compared the ChIP-chip and microarray data. Differentially expressed genes from the BrDSA situated adjacent to shared binding peaks from BRCA1 ChIP-chip were matched using gene accession number. Of the 24 695 transcripts covered on the ChIP-chip array 15 898 of their associated genes are also covered on the BrDSA (Supplementary Figure S5). Therefore, as not all of the transcripts identified within the promoter array were

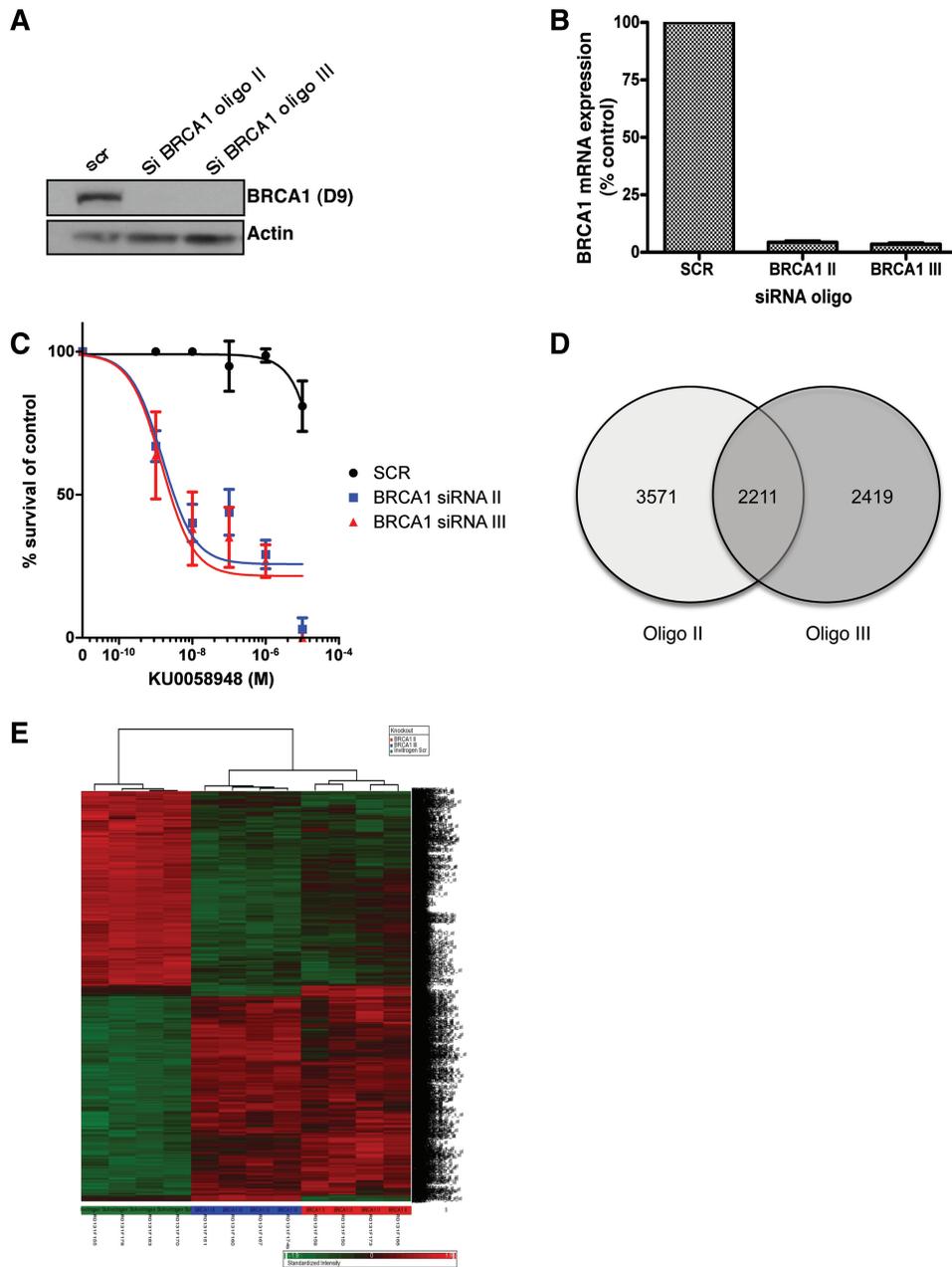


Figure 1. Microarray-based expression profiling. (A) Western blot and (B) qRT-PCR confirming siRNA inhibition of BRCA1 levels with two BRCA1 siRNA oligos (II and III) in MCF7 cells when compared with scrambled control (SCR). (C) Clonogenic assay demonstrating sensitivity to PARP-1 inhibitor (KU0058948) of cells treated with scrambled control (SCR) or BRCA1-specific siRNA oligos II or III. (D) Venn diagram of the probe set intersect after gene expression array analysis for each BRCA1 siRNA oligo; oligo II (left circle) or oligo III (right circle), in MCF7 cells. There are 2211 shared probe sets which correlate to 1294 genes. (E) Heat map from two-way hierarchical clustering analysis based on the 2211 common shared probe sets and 12 samples (4 per siRNA oligo).

present on the BrDSA expression array, we discounted the BRCA1 bound peaks that did not have corresponding gene probes on the BrDSA (Supplementary Figure S5). The number of BRCA1 bound ChIP-chip targets for comparison was therefore, reduced from 980 to 890. From 890 possible peaks (608 genes), we identified 65 probe sets (47 genes), which were common to both data sets (Figure 3A and Supplementary Figure S6). This was intriguing for two reasons; primarily, it suggested that a large proportion of BRCA1-regulated genes identified from BrDSA

expression profiling were modulated indirectly by BRCA1 loss. However, it also suggested that BRCA1 complexes found at the majority of promoters in unperturbed cells were non-functional in terms of gene expression modulation.

Characterization of direct BRCA1 transcriptional targets in unperturbed cells

To examine the first of these observations we sought to better understand the small subset of genes that were

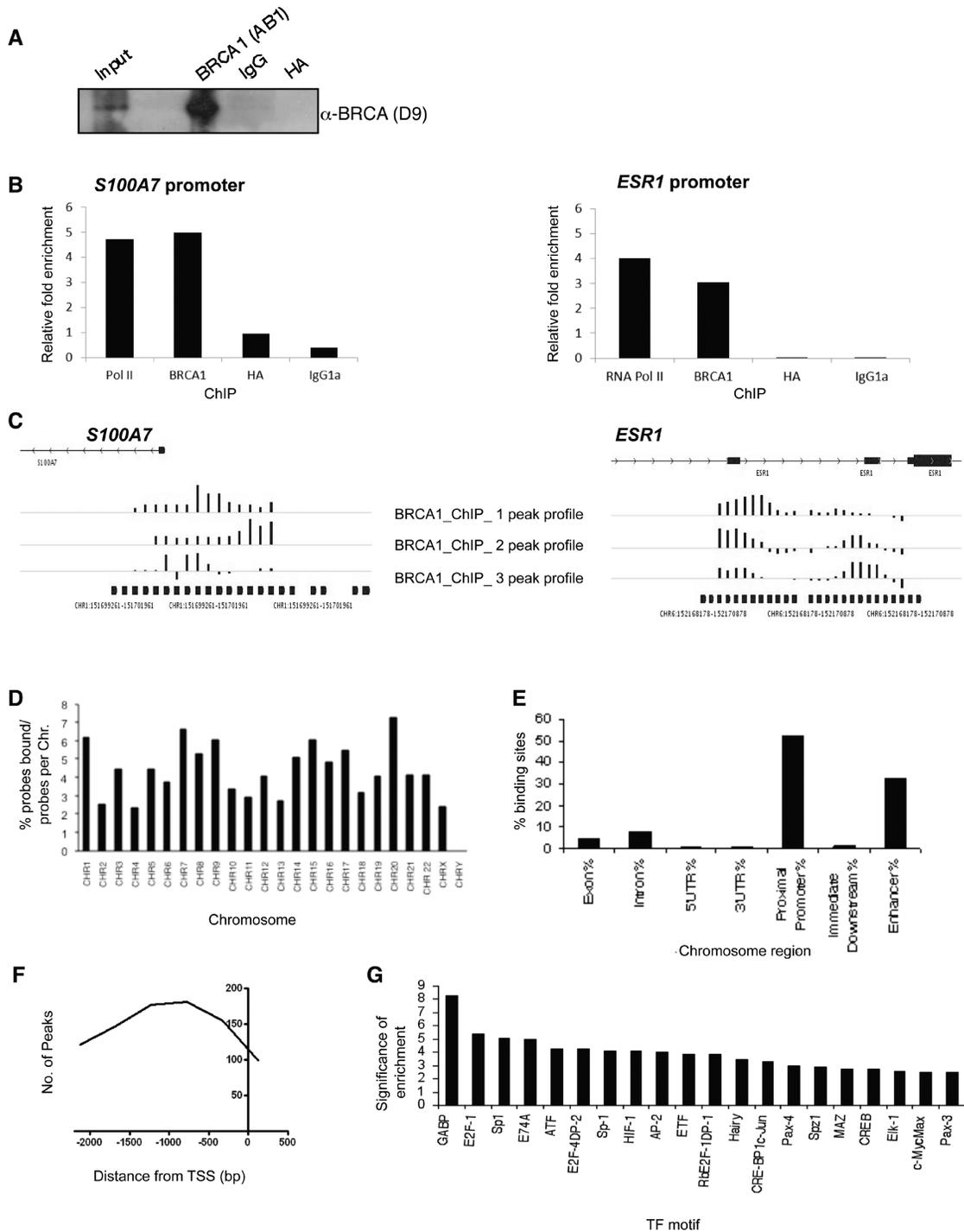


Figure 2. ChIP-chip expression profiling. (A) Western blot confirming immunoprecipitation of BRCA1 from MCF7 cells during ChIP with BRCA1-specific antibody AB1. (B) qRT-PCR for ChIP assays confirming BRCA1 recruitment to promoters of *S100A7* and *ESR1*. Amounts of immunoprecipitated DNA were normalized to inputs and reported relative to the amount obtained at a non-specific control region. Isotope matched IgG and HA antibodies were used as internal controls for each immunoprecipitation. (C) Binding peaks of the three independent BRCA1 ChIP samples from ChIP-chip data in MCF7 cells are shown for *S100A7* and *ESR1* probe sets. (D) Histogram demonstrating distribution of BRCA1 binding across the genome. The frequency of BRCA1 binding across chromosomes was calculated by dividing the number of probe sets per chromosome (data provided by NimbleGen) by the number of probe sets bound by BRCA1 with FDR <0.2. (E) Histogram demonstrating relative BRCA1 bound peak location with respect to chromosome region. (F) Chart demonstrating the significance of enrichment of the top 20 consensus TF binding motifs identified within peak coordinates of BRCA1 bound promoters where peak coordinates were within 500 bp of the TSS. Significance of enrichment represents those motifs which have hits which are significantly enriched in the ChIP regions with >2-fold change over hits within the genomic regions and binomial test $P < 1E-10$.

analysis (using the genes common to both the BrDSA and ChIP-chip array as a background gene list). DAVID (Database for Annotation, Visualisation and Integrated Discovery) analysis revealed that 12 of the 44 genes (27%) were involved in transcriptional regulation (Figure 3C). Therefore, this suggests that a major function of BRCA1 is the regulation of additional TFs, and may explain the large number of indirect targets identified through the microarray screen.

As the overlap between the BrDSA and the ChIP-chip array datasets is relatively small, it is possible that this overlap may have occurred by chance ($\chi^2 = 4.63$, 1 df, $P = 0.031$, z -score = -0.7170 , $P = 0.2367$). Therefore, in order to confirm that genes identified were indeed direct transcriptional targets of BRCA1, we performed ChIP and qRT-PCR analysis on a randomly selected panel of 14 (~30%) of the genes identified as well as the ChIP validation genes *ESR1* and *S100A7*. ChIP analysis demonstrated that BRCA1 was bound to the promoter region of all 16 genes tested. In addition, all of the genes identified were differentially regulated upon BRCA1 depletion in agreement with previously published data and data derived from the BrDSA expression array, suggesting that the methods used to identify the direct BRCA1 transcriptional targets were robust (Figure 3D and E, Supplementary Figure S7) (23,29).

Comparison of BRCA1 ChIP-chip data with gene expression profiles generated following cellular stress

As mentioned above, a surprising finding of this study was that, although BRCA1 was bound to the promoters of over 600 genes, only a subset displayed differential gene expression by microarray analysis following loss of BRCA1. We postulated that BRCA1 may be bound to these promoters primed to regulate transcription in response to BRCA1 responsive stimuli such as DNA damage or spindle poisons. In order to test this hypothesis, we compared our list of BRCA1 bound promoters to genes regulated in response to ionizing radiation (31), etoposide (29) or paclitaxel (32) (fold-change ≥ 1.7 , $P \leq 0.05$) from publicly available microarray expression data (Figure 4A and Supplementary Figure S8). This analysis revealed that different subsets of BRCA1 bound promoters were activated/repressed depending on the nature of the stimuli. For example, of the 36 genes that were identified as being BRCA1 promoter bound and differentially expressed following etoposide treatment, only a single gene was also found in the corresponding overlap from unperturbed cells (Figure 4B). This data is consistent with the hypothesis that BRCA1 is bound to many promoters in a non-functional complex that gets activated in response to stimuli such as etoposide treatment.

In order to confirm the *in-silico* data, we validated the expression of a subset of the BRCA1 promoter bound genes that were differentially regulated following etoposide treatment by qRT-PCR. Analysis revealed that BRCA1 functions to both repress and induce gene expression following etoposide treatment. For example, etoposide induced activation of *CCL4L2* is almost completely abrogated following siRNA-mediated inhibition of

endogenous BRCA1 (Figure 4C). Conversely, inhibition of endogenous BRCA1 results in a marked increase in etoposide-mediated induction of *PDE4DIP*, suggesting that BRCA1 functions to maintain repression of *PDE4DIP* following etoposide treatment. We also examined the protein expression of a subset of these genes and confirmed that BRCA1 depletion by siRNA exacerbated etoposide-induced induction (Figure 4D). We also assessed, using ChIP, if BRCA1 promoter occupancy is altered in response to etoposide treatment at a subset of these promoters (Figure 4E). In support of a model where BRCA1 is bound to promoters in both the absence and presence of DNA damage, BRCA1 occupancy of these eight representative promoters is unaltered after etoposide treatment.

Identification of novel BRCA1 interacting TFs

Finally, we sought to identify known and novel TFs that might be responsible for recruitment of BRCA1 by carrying out TF motif analysis on the BRCA1 bound regions of the 44 direct target genes identified in unperturbed cells. Using CEAS we ranked each consensus TF binding motif based on how many times the sequence appears within the given promoter, and on the significance of enrichment of the motif within that promoter compared with the entire human genome. This ranking was performed for all consensus TF motifs identified within BRCA1 bound regions on each target promoter. The 'hit' score for each TF motif was then weighted according to its rank within each individual promoter. We then plotted the combined hit score for each consensus TF motif to produce a graph representing the most highly ranked consensus TF motif across all of the direct BRCA1 target genes (Figure 5A). This analysis identified known BRCA1 interacting TFs such as STAT5A (33), TBP (4), CBP, CREB (34) and E2F (27). In addition, a number of novel putative BRCA1 interacting TFs were identified such as ZF5, AP2- α and Pax-2. BRCA1 co-immunoprecipitation experiments confirmed interactions between E2F-1 and BRCA1 but also interactions between BRCA1 and ZF5, AP2- α and Pax-2 (Figure 5B). Pax-4, also identified as a possible interacting TF in our analysis did not appear to interact with BRCA1. Unfortunately, although the Churchill (Churc1) TF ranked extremely highly in our analysis, antibodies were not available to this protein and thus, we could not test its ability to interact with BRCA1. In order to determine if these BRCA1 interacting TFs are indeed responsible for recruiting BRCA1 to specific promoters, we examined the ability of the two most highly ranked TFs from our search, AP2- α and ZF5 to recruit BRCA1 to the *NME1* and *PPM1D* promoters, respectively (these promoters were among those identified using CEAS to contain AP2- α and ZF5 binding sites) (Figure 5C). ChIP-qRT-PCR analysis revealed both AP2- α and BRCA1 bind the same region within the *NME1* promoter in control cells (siGFP), however, siRNA-mediated depletion of AP2- α results in loss of BRCA1 binding to the *NME1* promoter. Similarly, both BRCA1 and ZF5 bind the same region within the *PPM1D*

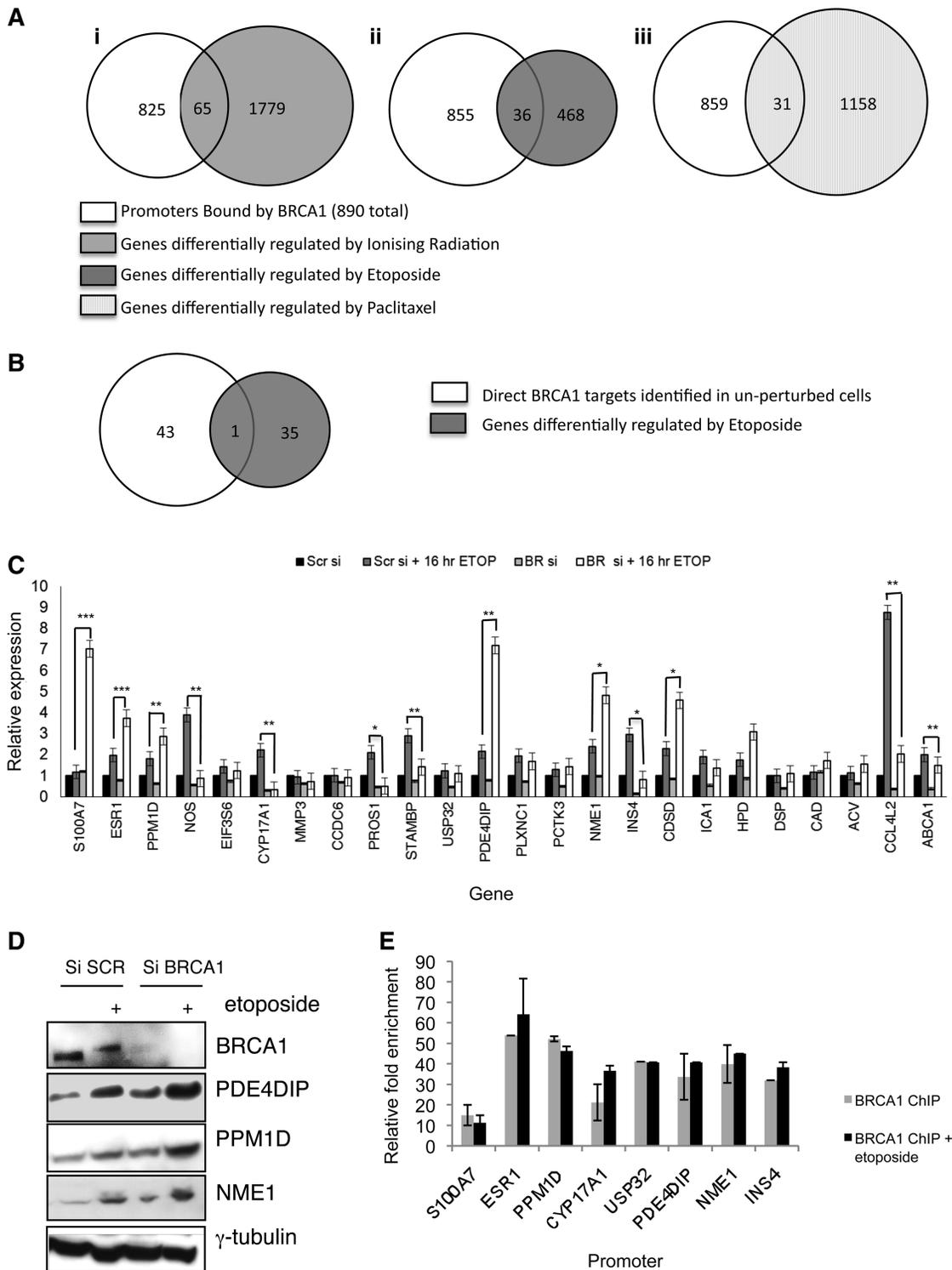


Figure 4. Comparison of BRCA1 ChIP-chip data with gene-expression profiles generated following cellular stress. **(A)** Venn diagrams displaying the intersection between transcripts identified as being differentially regulated following IR, etoposide and paclitaxel treatment with transcripts located in the promoters of the corresponding genes bound by BRCA1 from ChIP-chip analysis. **(B)** Venn diagram displaying the intersect between direct BRCA1 regulated targets in unperturbed cells with direct BRCA1 targets identified following etoposide treatment. **(C)** qRT-PCR showing fold change of expression between control and BRCA1-depleted cells upon etoposide treatment (5×10^{-6} M). The statistical significance of fold change upon treatment was analysed using the Student's *t*-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. **(D)** Western Blot analysis demonstrating the loss of etoposide induction on a panel of BRCA1 targets following siRNA-mediated inhibition of endogenous BRCA1 in HEK 293 cells. γ -tubulin was used as a loading control. **(E)** qRT-PCR of BRCA1 ChIP assays from unperturbed cells and etoposide treated (5×10^{-6} M) cells demonstrating that BRCA1 promoter occupancy at these promoters is unchanged in response to DNA damage. Amounts of immunoprecipitated DNA were normalized to inputs and reported relative to the amount obtained at a non-specific control region.

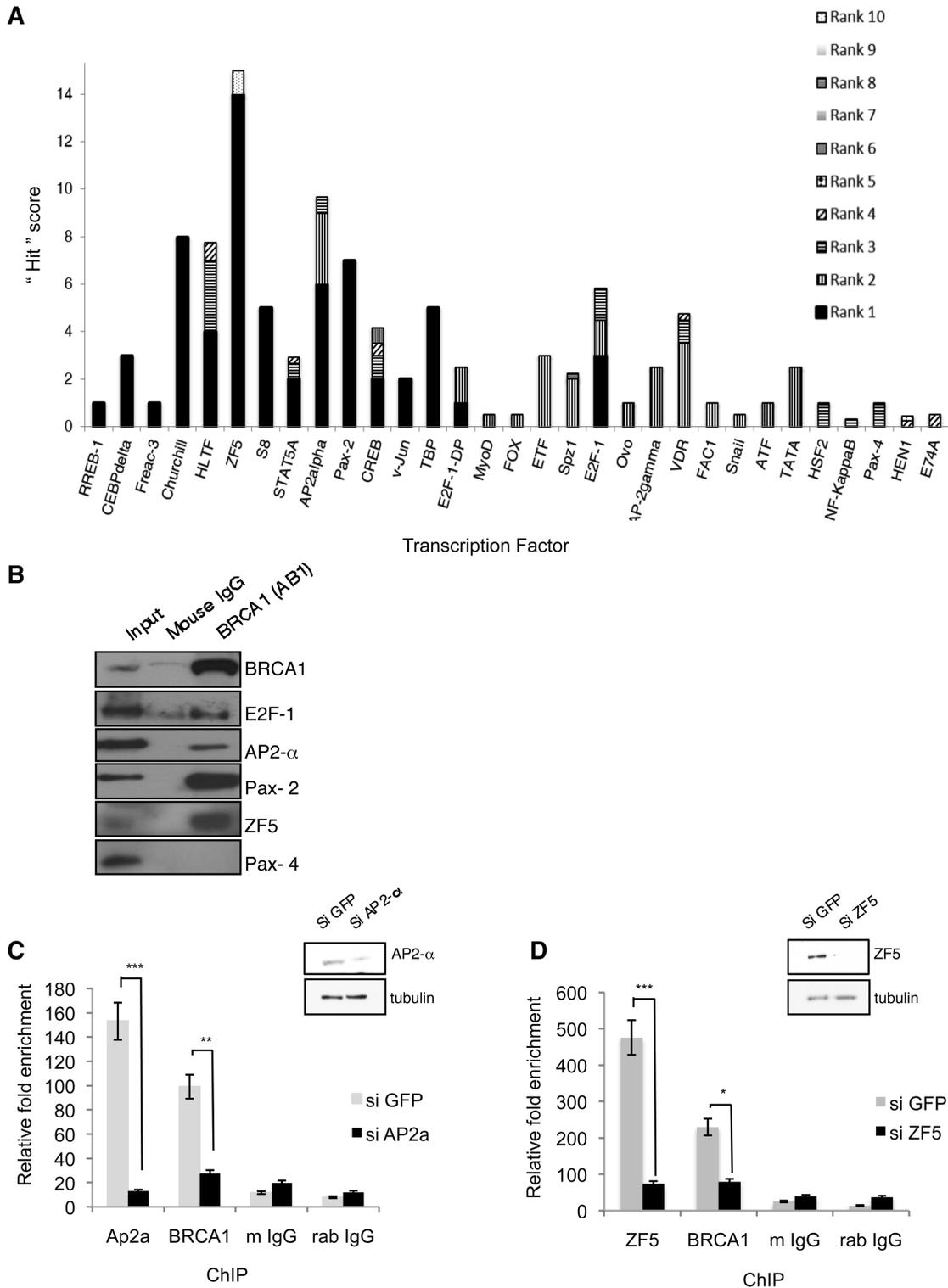


Figure 5. Identification of novel BRCA1 interacting TFs. (A) Histogram demonstrating significance of consensus TF binding motifs analysed by CEAS within the peak coordinates from the final 44 BRCA1 bound genes. Each TF identified was ranked on ‘hits’ per promoter and fold change. The greater the value, the more significant that TF is within the target promoters. (B) Co-immunoprecipitation demonstrating the association of endogenous BRCA1 with a panel of TFs in HEK 293 cells. (C) qRT-PCR of ChIP assay from cells treated with or without AP2- α -specific siRNA on the promoter of *NME1*, or (D) cells treated with or without ZF5-specific siRNA on the promoter of *PPM1D*, demonstrating that BRCA1 promoter occupancy is reduced in response to siRNA treatment. Amounts of immunoprecipitated DNA were normalized to inputs and reported relative to the amount obtained at a non-specific control region. The statistical significance of fold change upon siRNA treatment was analysed using the Student’s *t*-test; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

promoter; however, depletion of ZF5 results in loss of BRCA1 binding. These data suggest that both AP2- α and ZF5 are indeed functionally relevant BRCA1 interacting TFs, and supports a model, whereby novel BRCA1 interacting TFs mediate the recruitment/binding of BRCA1 to target gene promoters.

Discussion

This study aimed to investigate the role of BRCA1 in transcriptional regulation on a genomic scale by combining ChIP-chip and microarray based approaches. The overlap of the ChIP-chip and microarray data revealed a number of surprising findings. In the first instance, it indicated that of the 1294 differentially expressed genes identified by microarray, only 44 were identified as having BRCA1 bound at the promoter by ChIP-chip analysis. This would suggest that at least in unperturbed cells, the majority of differentially expressed genes identified by microarray represent indirect BRCA1 transcriptional targets. Gene ontology analysis revealed that ~30% of the direct BRCA1 targets identified were TFs providing a potential explanation for the large number of differentially expressed genes identified. Intriguingly, many of these TFs such as FOXM1 and TIMELESS, transcriptionally regulate genes tightly linked with BRCA1 associated pathways. FOXM1 regulates the G2-M cell-cycle checkpoint through transcriptional regulation of a number of genes required for mitotic progression. Additionally, FOXM1 induces the expression of a number of DNA repair genes and modulates cellular sensitivity to cisplatin and paclitaxel, all functions associated with BRCA1 (28,35,36). It is also likely that loss of BRCA1 expression may lead to transcriptional regulation through other more indirect mechanisms that are activated in response to BRCA1 regulated DNA damage repair pathways. Finally, it is possible that the stringent criteria we used to identify BRCA1 bound promoters may have led us to overlook some true BRCA1 bound regions. Indeed, we compared the 890 BRCA1 bound peaks identified in this study to a list of 29 BRCA1 bound promoters identified in the literature (Supplementary Figure S9). Of this list, 17 gene promoters (56%) were identified in at least one of the three BRCA1 ChIP-chip experiments. The lack of complete overlap of our list of BRCA1 bound promoters with previously published data is likely due to a number of factors. Among these is the disparity in sensitivity between ChIP array hybridization experiments versus ChIP-quantitative and ChIP-semi-quantitative PCR used in these studies. Additionally, the stringency used in this study reduced the number of BRCA1 bound regions identified, thereby, increasing the chance of missing true; albeit weak BRCA1 binding sites (37–39).

The other surprising observation from this study was the finding that the majority of BRCA1 promoter bound genes did not display transcriptional deregulation following BRCA1 depletion. This suggested that BRCA1 recruitment to these gene promoters was non-specific or that BRCA1 bound promoter complexes were primed to

respond to additional external stimuli. To test this hypothesis, we compared the ChIP-chip data with additional, publicly available, gene expression profiles generated, following treatment of cells with IR (Ionising Radiation), etoposide or paclitaxel. Overlapping of the data revealed that different subsets of BRCA1 promoter bound genes were differentially expressed following the different types of cellular stress. Moreover, comparison of the BRCA1 bound gene promoters regulated in response to IR with those regulated in response to etoposide revealed that 7 BRCA1 bound promoters, *ABCA1*, *MMP3*, *PDE4DIP*, *STAT1*, *RARRES1*, *TFF1* and *USP32* are commonly regulated in response to IR and etoposide treatment, suggesting that transcriptional regulation of these genes may represent a common BRCA1-mediated response to DNA damage. In contrast, comparing the BRCA1 bound gene promoters commonly regulated in response to IR and etoposide with those regulated in response to paclitaxel (a mitotic spindle poison) revealed only a single gene, *STAT1* commonly regulated in response to these agents. This is likely a reflection of the generalized stress response pathways activated by STAT1. Furthermore, comparison of the direct BRCA1 target genes identified in unperturbed cells with BRCA1 promoter bound genes transcriptionally regulated in cells treated with etoposide, revealed only a single common transcript, suggesting that BRCA1 regulates different subsets of genes in unperturbed cells and in response to different stimuli. Taken together, this data supports the hypothesis that BRCA1 is part of an early response transcriptional complex primed to respond differentially to a variety of different stimuli, such as genotoxic insult or mitotic spindle arrest.

We next demonstrated that a panel of the BRCA1 bound, etoposide regulated genes were altered by BRCA1 depletion in etoposide treated cells, again suggesting that BRCA1 regulates these genes in response to genotoxic stress. Importantly, BRCA1 binding to the promoters of these genes was not altered in response to etoposide treatment, suggesting that transcriptional regulation of these genes through BRCA1 is not regulated by alterations in BRCA1 promoter occupancy.

The mechanism through which BRCA1 promoter bound complexes are regulated in response to stimuli such as DNA damage and spindle checkpoint arrest remains unclear, however, it is likely to be through post-translational modification of BRCA1. Indeed, BRCA1 is phosphorylated by the ATM and ATR (ATM and Rad3 related kinase) kinases at a number of serine residues following etoposide and IR-mediated DNA damage, and by Chk2 in response to spindle poisons such as paclitaxel (40,41). In addition, given the large number of different BRCA1 containing protein complexes formed in response to DNA damage, it is possible that phosphorylation of BRCA1 may regulate its ability to form different transcriptional at BRCA1 bound promoters. It is also unclear how different stimuli impact on BRCA1s ability to transactivate or repress various genes. For example, BRCA1, in complex with ZBRK1 and CtIP TFs represses the expression of growth arrest and DNA damage gene *GADD45 α* in unperturbed cells. Meanwhile, a separate complex, consisting of BRCA1 and NF-YA recruited by

Oct-1, is responsible for GADD45 α -mediated activation in response to DNA damage (42,43). Exactly what role BRCA1 plays in these different complexes remains unknown, however, the finding that BRCA1 is required for both the ZBRK1-mediated repression of GADD45 α , as well as, the Oct-1-mediated activation of this gene, suggests that perhaps BRCA1 may facilitate some sort of switching mechanism in response to DNA damage. It is possible that BRCA1 provides a scaffolding-like function on these promoters functioning as a substrate for the recruitment of co-activator or co-repressor proteins upon cellular stress, which is potentially regulated by BRCA1 phosphorylation. This is analogous to the roles played by Hot1 and Smp1 proteins in *Saccharomyces Cerevisiae*, facilitating MAP kinase (Mitogen-activated protein kinase) signalling induced transcription previously (44).

Since little is known about BRCA1 promoter binding on a genomic scale, we analysed the specific regions bound by BRCA1 in the 44 direct BRCA1 target genes identified in unperturbed cells. CEAS analysis revealed that BRCA1 preferentially bound within the proximal promoter region consistent with a role in the direct regulation of these transcripts. We also attempted to determine if any common conserved BRCA1 binding motif existed within bound regions. We did not identify any conserved sequence across BRCA1 binding regions including the previously reported BRCA1 consensus binding site, TTC(G/T)GTTG (45). This suggested that there is no *in vivo* BRCA1 consensus binding motif and that it is probable that, BRCA1 interacts with promoters via interactions with multiple TFs. To identify potential TFs that may be responsible for BRCA1 recruitment, we identified possible consensus binding motifs within the promoters of the 44 genes differentially expressed following BRCA1 depletion which were also promoter bound by BRCA1. This identified a number of known BRCA1 interacting TFs such as STAT5A (33), TBP (4), CBP, CREB (34) and E2F (27) which have all been demonstrated to alter the transcriptional activity of BRCA1. This also identified a number of novel putative BRCA1 interacting TFs such as ZF5, AP2- α , Pax-2 and Churc1 which, with the exception of Churc1, we confirmed as BRCA1 interactors. We confirmed that both AP2- α and ZF5 are required for BRCA1 promoter binding at target gene promoters, suggesting that many of the novel BRCA1 interacting TFs identified are also likely required for BRCA1 binding at specific target gene promoters. AP2- α , ZF5 and Pax-2, all function in the regulation of distinct transcriptional programs. ZF5 is a kruppel-type TF which has been shown to regulate the expression of the fragile-X causative gene *FMRI* (46). In addition, binding sites for ZF5 are enriched in the genetic promoters of clock-regulated genes, as have binding sites for the known BRCA1 interacting TFs; E2F, AP-1 and NF-Y, SP1 and Oct-1 (14,15,47). ZF5 binding sites are also enriched in the promoters of Hermansky-Pudlak syndrome genes *HPS3*, *HPS5* and *HPS6* (48); interestingly, this study also found enrichment of Churc1 binding sites within the promoters of these genes. Taken together, this suggests that BRCA1, Churc1, ZF5 and the other BRCA1 interacting TFs

mentioned above may exist as a single and/or multiple complex(es) involved in the transcriptional regulation of various genes. AP2- α is involved in the transcriptional regulation of many genes such as those involved in regulation of the G2-M checkpoint, including *Cyclin-B*, and has been reported to function as a co-repressor for Myc-activated genes (49,50). Consistent with this, BRCA1 is thought to regulate the G2-M checkpoint, in-part, by repressing *Cyclin-B* (51). In addition, we have previously shown that BRCA1 and c-Myc function together in a transcriptional repression complex (29). In contrast, Pax-2 is predominantly associated with the transcriptional regulation of developmental genes involved in many different pathways such as hedgehog signalling.

This study has identified a number of interesting findings regarding the role played by BRCA1 in transcriptional regulation at a genome-wide level. It is clear that although deregulation of BRCA1 has a profound impact on transcriptional regulation, much of this is indirect. This study indicates that BRCA1 resides on many promoters in an inactive complex that is primed for response to various stimuli such as DNA damage and mitotic spindle poisons. Given the differential phosphorylation patterns of BRCA1 in response to various DNA damaging agents and mitotic spindle poisons, it is possible that BRCA1 facilitates the fine-tuning of transcriptional activation and/or repression in response to different external stimuli, through phosphorylation-mediated assembly of various transcriptional complexes.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

FUNDING

Cancer Research UK (C538/A8132 to J.J.G., K.I.S. and D.P.H.); Department of Education and Learning, Northern Ireland (to J.K.B.); Medical Research Council (G0700754 to S.M.). Funding for open access charge: Cancer Research UK.

Conflict of interest statement. D. Paul Harkin is an employee and stock holder of Almac Diagnostics Ltd, the company that provided expression array services used in this study.

REFERENCES

- Monteiro,A.N., August,A. and Hanafusa,H. (1996) Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc. Natl Acad. Sci. USA*, **93**, 13595–13599.
- Humphrey,J.S., Salim,A., Erdos,M.R., Collins,F.S., Brody,L.C. and Klausner,R.D. (1997) Human BRCA1 inhibits growth in yeast: potential use in diagnostic testing. *Proc. Natl Acad. Sci. USA*, **94**, 5820–5825.
- Anderson,S.F., Sclegel,B.P., Nakajima,T., Wolpin,E.S. and Parvin,J.D. (1998) BRCA1 protein is linked to the RNA polymerase II holoenzyme complex via RNA helicase A. *Nat. Genet.*, **19**, 254–256.
- Scully,R., Anderson,S.F., Chao,D.M., Wei,W., Ye,L., Young,R.A., Livingston,D.M. and Parvin,J.D. (1997) BRCA1

- is a component of the RNA polymerase II holoenzyme. *Proc. Natl Acad. Sci. USA*, **94**, 5605–5610.
5. Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S.J. and Qin, J. (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Genes Dev.*, **14**, 927–939.
 6. Fabbro, M., Savage, K., Hobson, K., Deans, A.J., Powell, S.N., McArthur, G.A. and Khanna, K.K. (2004) BRCA1-BARD1 complexes are required for p53Ser-15 phosphorylation and a G1/S arrest following ionizing radiation-induced DNA damage. *J. Biol. Chem.*, **279**, 31251–31258.
 7. Fan, S., Ma, Y.X., Wang, C., Yuan, R.-q., Meng, Q., Wang, J.-A., Erdos, M., Goldberg, I.D., Webb, P., Kushner, P.J. *et al.* (2001) Role of direct interaction in BRCA1 inhibition of estrogen receptor activity. *Oncogene*, **20**, 77–87.
 8. Zheng, L., Annab, L.A., Afshari, C.A., Lee, W.H. and Boyer, T.G. (2001) BRCA1 mediates ligand-independent transcriptional repression of the estrogen receptor. *Proc. Natl Acad. Sci. USA*, **98**, 9587–9592.
 9. Kennedy, R.D., Gorski, J.J., Quinn, J.E., Stewart, G.E., James, C.R., Moore, S., Mulligan, K., Emberley, E.D., Lioe, T.F., Morrison, P.J. *et al.* (2005) BRCA1 and c-Myc associate to transcriptionally repress psoriasis, a DNA damage-inducible gene. *Cancer Res.*, **65**, 10265–10272.
 10. Hosey, A.M., Gorski, J.J., Murray, M.M., Quinn, J.E., Chung, W.Y., Stewart, G.E., James, C.R., Farragher, S.M., Mulligan, J.M., Scott, A.N. *et al.* (2007) Molecular basis for estrogen receptor alpha deficiency in BRCA1-linked breast cancer. *J. Natl Cancer Inst.*, **99**, 1683–1694.
 11. Gorski, J.J., James, C.R., Quinn, J.E., Stewart, G.E., Staunton, K.C., Buckley, N.E., McDyer, F.A., Kennedy, R.D., Wilson, R.H., Mullan, P.B. *et al.* (2010) BRCA1 transcriptionally regulates genes associated with the basal-like phenotype in breast cancer. *Breast Cancer Res. Treat.*, **122**, 721–731.
 12. Lamber, E.P., Horwitz, A.A. and Parvin, J.D. BRCA1 represses amphiregulin gene expression. *Cancer Res.*, **70**, 996–1005.
 13. Harte, M.T., O'Brien, G.J., Ryan, N.M., Gorski, J.J., Savage, K.I., Crawford, N.T., Mullan, P.B. and Harkin, D.P. (2010) BRD7, a subunit of SWI/SNF complexes, binds directly to BRCA1 and regulates BRCA1-dependent transcription. *Cancer Res.*, **70**, 2538–2547.
 14. Mullan, P.B., Quinn, J.E. and Harkin, D.P. (2006) The role of BRCA1 in transcriptional regulation and cell cycle control. *Oncogene*, **25**, 5854–5863.
 15. Savage, K.I. and Harkin, D.P. (2009) BRCA1 and BRCA2: Role in the DNA Damage Response, Cancer Formation and Treatment. In Shiloh, Y. and Khanna, K.K. (eds), *The DNA Damage Response: Implications on Cancer Formation and Treatment*, Vol. 1. Springer, Dordrecht, Heidelberg, London, New York, pp. 415–443.
 16. Chen, G.-C., Guan, L.-S., Yu, J.-H., Li, G.-C., Kim, H.-R.C. and Wang, Z.-Y. (2001) Rb-associated protein 46 (RbAp46) inhibits transcriptional transactivation mediated by BRCA1. *Biochem. Biophys. Res. Comm.*, **284**, 507–514.
 17. Yarden, R.I. and Brody, L.C. (1999) BRCA1 interacts with components of the histone deacetylase complex. *Proc. Natl Acad. Sci. USA*, **96**, 4983–4988.
 18. Oishi, H., Kitagawa, H., Wada, O., Takezawa, S., Tora, L., Kouzu-Fujita, M., Takada, I., Yano, T., Yanagisawa, J. and Kato, S. (2006) An hGCN5/TRRAP histone acetyltransferase complex co-activates BRCA1 transactivation function through histone modification. *J. Biol. Chem.*, **281**, 20–26.
 19. Harte, M.T., O'Brien, G.J., Ryan, N.M., Gorski, J.J., Savage, K.I., Crawford, N.T., Mullan, P.B. and Harkin, D.P. BRD7, a subunit of SWI/SNF complexes, binds directly to BRCA1 and regulates BRCA1-dependent transcription. *Cancer Res.*, **70**, 2538–2547.
 20. Farmer, H., McCabe, N., Lord, C.J., Tutt, A.N., Johnson, D.A., Richardson, T.B., Santarosa, M., Dillon, K.J., Hickson, I., Knights, C. *et al.* (2005) Targeting the DNA repair defect in BRCA mutant cells as a therapeutic strategy. *Nature*, **434**, 917–921.
 21. Bryant, H.E., Schultz, N., Thomas, H.D., Parker, K.M., Flower, D., Lopez, E., Kyle, S., Meuth, M., Curtin, N.J. and Helleday, T. (2005) Specific killing of BRCA2-deficient tumours with inhibitors of poly(ADP-ribose) polymerase. *Nature*, **434**, 913–917.
 22. Weng, L., Dai, H., Zhan, Y., He, Y., Stepaniants, S.B. and Bassett, D.E. (2006) Rosetta error model for gene expression analysis. *Bioinformatics*, **22**, 1111–1121.
 23. Hosey, A.M., Gorski, J.J., Murray, M.M., Quinn, J.E., Chung, W.Y., Stewart, G.E., James, C.R., Farragher, S.M., Mulligan, J.M., Scott, A.N. *et al.* (2007) Molecular basis for estrogen receptor {alpha} deficiency in BRCA1-linked breast cancer. *J. Natl Cancer Inst.*, **99**, 1683–1694.
 24. Gentleman, R.C., Carey, V.J., Bates, D.M., Bolstad, B., Dettling, M., Dudoit, S., Ellis, B., Gautier, L., Ge, Y., Gentry, J. *et al.* (2004) Bioconductor: open software development for computational biology and bioinformatics. *Genome Biol.*, **5**, R80.
 25. Ameur, A., Rada-Iglesias, A., Komorowski, J. and Wadelius, C. (2009) Identification of candidate regulatory SNPs by combination of transcription-factor-binding site prediction, SNP genotyping and haploChIP. *Nucleic Acids Res.*, **37**, e85.
 26. Ernst, P. and Smale, S.T. (1995) Combinatorial regulation of transcription. I: General aspects of transcriptional control. *Immunity*, **2**, 311–319.
 27. De Siervi, A., De Luca, P., Byun, J.S., Di, L.J., Fufa, T., Haggerty, C.M., Vazquez, E., Muiola, C., Longo, D.L. and Gardner, K. (2010) Transcriptional autoregulation by BRCA1. *Cancer Res.*, **70**, 532–542.
 28. Tan, Y., Raychaudhuri, P. and Costa, R.H. (2007) Chk2 mediates stabilization of the FoxM1 transcription factor to stimulate expression of DNA repair genes. *Mol. Cell Biol.*, **27**, 1007–1016.
 29. Kennedy, R.D., Gorski, J.J., Quinn, J.E., Stewart, G.E., James, C.R., Moore, S., Mulligan, K., Emberley, E.D., Lioe, T.F., Morrison, P.J. *et al.* (2005) BRCA1 and c-Myc associate to transcriptionally repress psoriasis, a DNA damage-inducible gene. *Cancer Res.*, **65**, 10265–10272.
 30. Li, H., Lee, T.-H. and Avraham, H. (2002) A novel tricomplex of BRCA1, Nmi, and c-Myc inhibits c-Myc-induced human telomerase reverse transcriptase gene (hTERT) promoter activity in breast cancer. *J. Biol. Chem.*, **277**, 20965–20973.
 31. Kumaraswamy, S., Chinnaiyan, P., Shankavaram, U.T., Lu, X., Camphausen, K. and Tofilon, P.J. (2008) Radiation-induced gene translation profiles reveal tumor type and cancer-specific components. *Cancer Res.*, **68**, 3819–3826.
 32. Hernandez-Vargas, H., von Kobbe, C., Sanchez-Estevéz, C., Julian-Tendero, M., Palacios, J. and Moreno-Bueno, G. (2007) Inhibition of paclitaxel-induced proteasome activation influences paclitaxel cytotoxicity in breast cancer cells in a sequence-dependent manner. *Cell Cycle*, **6**, 2662–2668.
 33. Vidarsson, H., Mikaelsson, E.K., Rafnar, T., Bertwistle, D., Ashworth, A., Eyfjord, J.E. and Valgeirsdottir, S. (2002) BRCA1 and BRCA2 bind Stat5a and suppress its transcriptional activity. *FEBS Lett.*, **532**, 247–252.
 34. Neish, A.S., Anderson, S.F., Schlegel, B.P., Wei, W. and Parvin, J.D. (1998) Factors associated with the mammalian RNA polymerase II holoenzyme. *Nucleic Acids Res.*, **26**, 847–853.
 35. Kwok, J.M., Peck, B., Monteiro, L.J., Schwenen, H.D., Millour, J., Coombes, R.C., Myatt, S.S. and Lam, E.W. FOXM1 confers acquired cisplatin resistance in breast cancer cells. *Mol. Cancer Res.*, **8**, 24–34.
 36. Carr, J.R., Park, H.J., Wang, Z., Kiefer, M.M. and Raychaudhuri, P. FoxM1 mediates resistance to herceptin and paclitaxel. *Cancer Res.*, **70**, 5054–5063.
 37. Fernandez-Ramires, R., Sole, X., De Cecco, L., Llort, G., Cazorla, A., Bonifaci, N., Garcia, M.J., Caldes, T., Blanco, I., Gariboldi, M. *et al.* (2009) Gene expression profiling integrated into network modelling reveals heterogeneity in the mechanisms of BRCA1 tumorigenesis. *Br. J. Cancer*, **101**, 1469–1480.
 38. Gorski, J.J., James, C.R., Quinn, J.E., Stewart, G.E., Staunton, K.C., Buckley, N.E., McDyer, F.A., Kennedy, R.D., Wilson, R.H., Mullan, P.B. *et al.* (2010) BRCA1 transcriptionally regulates genes associated with the basal-like phenotype in breast cancer. *Breast Cancer Res. Treat.*, **122**, 721–731.
 39. Lamber, E.P., Horwitz, A.A. and Parvin, J.D. (2010) BRCA1 represses amphiregulin gene expression. *Cancer Res.*, **70**, 996–1005.

40. Chabalier-Taste,C., Racca,C., Dozier,C. and Larminat,F. (2008) BRCA1 is regulated by Chk2 in response to spindle damage. *Biochim. Biophys. Acta.*, **1783**, 2223–2233.
41. Gatei,M., Zhou,B.B., Hobson,K., Scott,S., Young,D. and Khanna,K.K. (2001) Ataxia telangiectasia mutated (ATM) kinase and ATM and Rad3 related kinase mediate phosphorylation of Brcal at distinct and overlapping sites. In vivo assessment using phospho-specific antibodies. *J. Biol. Chem.*, **276**, 17276–17280.
42. Fan,W., Jin,S., Tong,T., Zhao,H., Fan,F., Antinore,M.J., Rajasekaran,B., Wu,M. and Zhan,Q. (2002) BRCA1 regulates GADD45 through its interactions with the OCT-1 and CAAT motifs. *J. Biol. Chem.*, **277**, 8061–8067.
43. Zhao,H., Jin,S., Fan,F., Fan,W., Tong,T. and Zhan,Q. (2000) Activation of the transcription factor Oct-1 in response to DNA damage. *Cancer Res.*, **60**, 6276–6280.
44. Weake,V.M. and Workman,J.L. Inducible gene expression: diverse regulatory mechanisms. *Nat. Rev. Genet.*, **11**, 426–437.
45. Cable,P.L., Wilson,C.A., Calzone,F.J., Rauscher,F.J. III, Scully,R., Livingston,D.M., Li,L., Blackwell,C.B., Futreal,P.A. and Afshari,C.A. (2003) Novel consensus DNA-binding sequence for BRCA1 protein complexes. *Mol. Carcinog.*, **38**, 85–96.
46. Orlov,S.V., Kuteykin-Teplyakov,K.B., Ignatovich,I.A., Dizhe,E.B., Mirgorodskaya,O.A., Grishin,A.V., Guzhova,O.B., Prokhortchouk,E.B., Guliy,P.V. and Perevozchikov,A.P. (2007) Novel repressor of the human FMR1 gene - identification of p56 human (GCC)(n)-binding protein as a Kruppel-like transcription factor ZF5. *FEBS J.*, **274**, 4848–4862.
47. El-Tanani,M.K., Campbell,F.C., Crowe,P., Erwin,P., Harkin,D.P., Pharoah,P., Ponder,B. and Rudland,P.S. (2006) BRCA1 suppresses osteopontin-mediated breast cancer. *J. Biol. Chem.*, **281**, 26587–26601.
48. Stanescu,H., Wolfsberg,T.G., Moreland,R.T., Ayub,M.H., Erickson,E., Westbroek,W., Huizing,M., Gahl,W.A. and Helip-Wooley,A. (2009) Identifying putative promoter regions of Hermansky-Pudlak syndrome genes by means of phylogenetic footprinting. *Ann. Hum. Genet.*, **73**, 422–428.
49. Cogswell,J.P., Godlevski,M.M., Bonham,M., Bisi,J. and Babiss,L. (1995) Upstream stimulatory factor regulates expression of the cell cycle-dependent cyclin B1 gene promoter. *Mol Cell Biol.*, **15**, 2782–2790.
50. Trembley,J.H., Chen,Z., Rodrigues,C.M., Kren,B.T., Steer,C.J. (2000) Genomic organization and promoter characterization of the rat cyclin B1 gene. *Gene.*, **255**, 93–104.
51. MacLachlan,T.K., Somasundaram,K., Sgagias,M., Shifman,Y., Muschel,R.J., Cowan,K.H. and El-Deiry,W.S. (2000) BRCA1 effects on the cell cycle and the DNA damage response are linked to altered gene expression. *J. Biol. Chem.*, **275**, 2777–2785.