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Characterisation of gene expression profiles of yeast cells expressing BRCA1 missense variants

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ABSTRACT

Germline mutations in breast cancer susceptibility gene 1 (BRCA1) confer high risk of developing breast and ovarian cancers. Even though most BRCA1 cancer-predisposing mutations produce a non-functional truncated protein, 5–10% of them cause single amino acid substitutions. This second type of mutations represents a useful tool for examining BRCA1 molecular functions. Human BRCA1 inhibits cell proliferation in transformed *Saccharomyces cerevisiae* cells and this effect is abolished by disease-associated mutations in the BRCT domain. Moreover, BRCA1 mutations located both inside and outside the BRCT domain may induce an increase in the homologous recombination frequency in yeast cells. Here we present a microarray analysis of gene expression induced in yeast cells transformed with five BRCA1 missense variants, in comparison with gene expression induced by wild-type BRCA1. Data analysis was performed by grouping the BRCA1 variants into three sets: Recombination (R)-set (Y179C and S1164I), Recombination and Proliferation (RP)-set (I1766S and M1775R) and Proliferation (P)-set (A1789T), according to their effects on yeast cell phenotype. We found 470, 740 and 1136 differentially expressed genes in R-, P- and RP-set, respectively. Our results point to some molecular mechanisms critical for the control of cell proliferation and of genome integrity providing support to a possible pathogenic role of the analysed mutations. They also confirm that yeast, despite the absence of a BRCA1 homologue, represents a valid model system to examine BRCA1 molecular functions, as the molecular pathways activated by BRCA1 variants are conserved in humans.

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1. Introduction

Germline mutations in the breast cancer susceptibility gene 1, BRCA1, predispose to breast and ovarian cancers.¹

Human BRCA1 encodes a full-length protein of 1863 amino acids containing some known functional domains: a highly conserved N-terminal RING finger domain, two nuclear localisation signals, an 'SQ' cluster, a branched DNA-binding domain and C-terminal BRCT domains.²

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BRCA1 appears to coordinate multiple activities linked to the maintenance of genomic integrity and to function as a tumour suppressor, through the direct or indirect interaction with a large number of molecules, including tumour suppressors, oncogenes, DNA damage repair proteins, cell cycle regulators, transcriptional activators and repressors.³ BRCA1 plays a role in homologous recombination (HR) during both mitosis and meiosis and regulates the homology-directed DNA repair and crossing over.^{4,5} It is also involved in non-homologous end-joining⁶ and mismatch repair, both in response to DNA damage⁷ and during chromosome segregation.⁸ BRCA1 growth suppressor function in mammalian cells depends at least in part on its interaction with retinoblastoma protein (RB) but also on interaction with a series of other cell cycle-related proteins such as p53 and p21.^{9,10}

Most pathogenic BRCA1 mutations originate a truncated protein, but a number of missense mutations, whose role in the disease is often difficult to ascertain, have also been detected in hereditary breast cancer patients. An almost complete list of genetic variants detected till now in BRCA1 and BRCA2 genes is reported in BIC (Breast Cancer Information Core) database (<http://research.nhgri.nih.gov/bic/>).

Saccharomyces cerevisiae has been shown to represent a good system to discriminate between pathogenic and polymorphic missense mutations located in BRCA1 BRCT domains, as inactivating mutations within BRCT domains abolish BRCA1 ability to inhibit yeast cell proliferation.¹¹ To elucidate the BRCA1 BRCT domain effects on gene expression, Skibbens et al.¹² compared yeast cells transformed with vector or with vector containing the BRCA1 BRCT domain by microarray analysis. Even though their analysis was limited to the BRCT domain, they showed that the yeast model is also useful to elucidate molecular aspects of BRCA1 function.

Caligo et al.,¹³ as well, have shown that some BRCA1 missense mutations isolated from patients' breast cancers and located outside the BRCT domains, in scattered positions along the sequence, induce an increase in HR. This suggests that yeast may represent a model for the study of both BRCA1 molecular functions: growth suppression and maintenance of genome integrity.

Here we present a study in which gene expression induced in *S. cerevisiae* by each of five BRCA1 missense variants was compared by microarray analysis to that induced by wild-type BRCA1. All five variants had been isolated from familial breast cancers and had induced a phenotypic change in yeast cells, either on proliferation, or on HR or both.¹³ three carried mis-

sense mutations within the BRCT domains, and two outside the BRCT domains.

2. Materials and methods

2.1. Yeast strain and BRCA1 missense variants

We used the diploid strain RS112 of *S. cerevisiae* (from Robert Schiestl, UCLA, Los Angeles, CA, USA) transformed with the vector YCp GAL::BRCA1 which contains the human BRCA1 gene under the control of galactose inducible promoter GAL1p (obtained from Craig Bennett, Duke University, Durham, NC, USA) or with the derivative vectors carrying the following BRCA1 missense variants: Y179C, S1164I, I1766S, M1775R and A1789T.¹³ Except for M1775R that had been described as deleterious by a transcriptional activation assay¹⁴ and, more recently, by a multi-modal approach,¹⁵ all variants were either not reported or described as missense variants of unknown pathological significance in BIC database. However, in silico analyses with Sorting Intolerant From Tolerant (SIFT) (<http://blocks.fhcrc.org/sift/SIFT.html>) and Polymorphism Phenotyping (PolyPhen) (<http://tux.embl-heidelberg.de/ramensky/polyphen.cgi>) show that all variants probably inactivate protein function.¹³ The I1766S variant has been reported as deleterious in one paper,¹⁶ while the Y179C has previously been studied without reaching any conclusive result,^{17,18} S1164I and A1789T had never been studied before the work of Caligo and colleagues, 2009.¹³

Concerning the effects on yeast cell phenotype, only the mutations in the BRCT domains (I1766S, M1775R and A1789T) reverted the growth suppression (small colony) phenotype,¹³ typically observed in yeast cells transformed with wild-type BRCA1.¹¹ Two of them, I1766S and M1775R, also induced HR. As expected, the two variants, Y179C and S1164I, mapping outside the BRCT domains, did not revert the small colony phenotype, but induced HR.¹³ Table 1 resumes all five variants' features.

2.2. Induction of BRCA1 expression

The expression of BRCA1 was induced as follows: stable transformants obtained as reported by Caligo et al.¹³ were pre-grown in 10–20 ml of glucose medium for 24 h at 30 °C. Then, cell pellets were washed in water and were split into two aliquots: one was inoculated in 20 ml of glucose and the other

Table 1 – Description of the analysed BRCA1 missense variants.

Variant	Nucleotide	BRCA1 functional domain	Predicted pathogenicity ^a	Reported pathogenicity	Small colony phenotype assay	Homologous recombination induction
Y179C	c.655A > G	cMyc interaction domain	Damaging	Neutral ^{16,17}	–	+
S1164I	c.3610G > T	DNA-binding domain	Damaging	Never studied before	–	+
I1766S	c.5416T > G	BRCT domain	Damaging	Deleterious ¹⁵	+	+
M1775R	c.5443T > G	BRCT domain	Damaging	Deleterious ^{13,14}	+	+
A1789T	c.5484G > A	BRCT domain	Probable	Never studied before	+	–

a Based on SIFT, PolyPhen and BIC.

was inoculated in 20 ml of galactose medium. The cultures were incubated at 30 °C for 24 h under constant shaking. Thereafter, cells were washed twice in sterile water and were immediately used to extract RNA or stored at –20 °C.

The expression of BRCA1 protein was assessed by Western blot analysis.¹³ The observed phenotypes (proliferation or HR) were independent from the level of expression of BRCA1 variants.¹³

2.3. Isolation of RNA

Total RNA extraction and DNase treatment were performed with the MasterPure™ Yeast RNA Purification Kit (Epicentre Biotechnologies, Madison, WI, USA), according to the manufacturer's instructions. The RNA was further cleaned using the chromatographic system RNeasy MinElute Cleanup (Qia-gen, Valencia, CA, USA).

The concentration and purity of total RNA were measured by 260 nm UV absorption and by 260/280 ratios, respectively, by using NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc. Wilmington, Del, USA): all RNAs displayed a 260/280 OD ratio > 1.9.

The RNA integrity was verified by electrophoresis on a 1.2% agarose-formaldehyde gel.

2.4. Microarray hybridisation

One microgram of each RNA sample was amplified and dye-labelled with Alexa 647 (red) or Alexa 555 (green) (Invitrogen, Carlsbad, CA, USA) with the Amino Allyl MessageAmp™ II aRNA Amplification Kit (Ambion, Austin, TX, USA).

The Alexa 647 and Alexa 555 dye incorporation rates were measured by UV absorption at 647 nm and 555 nm, respectively. Both fluorophores showed a comparable efficiency of incorporation ranging between 5 and 6 dye molecules per 100 nucleotides. Afterwards, 250 µl of hybridisation mix containing 0.25 µg of Alexa 555-labelled amplified RNA (corresponding to 35–40 picomoles of Alexa 555 dye), 0.25 µg of Alexa 647-labelled amplified RNA (corresponding to 35–40 picomoles of Alexa 647 dye), 25 µl of 10X control targets, 5 µl of 25X fragmentation buffer and 125 µl of 2X hybridisation buffer (the three latter ones from the In situ hybridisation kit plus, Agilent Technologies, Palo Alto, CA, USA) was hybridised to each array on Yeast Oligo 2×11k Microarrays (Agilent Technologies, Palo Alto, CA, USA). Each slide contains 2 arrays with

11,000 60-mer oligonucleotide probes representing 6256 Open Reading Frame (ORF) of *S. cerevisiae* S288C strain. The array hybridisation was performed at 60 °C in an oven (Agilent Technologies, Palo Alto, CA, USA) for 17 h under constant rotation.

After hybridisation, the arrays were washed consecutively in 6X SSC, 0.005% TritonX-102 (In situ Hybridisation kit Plus, Agilent Technologies, Palo Alto, CA, USA) solution for 10 min at room temperature and in 0.1X SSC, 0.005% Triton X-102 solution for 5 min on ice and were air dried.

2.5. Experimental design

Microarray experiments were performed by using a dye swap 'reference design'.

The RNA from yeast cells transformed with each BRCA1 variant (BRCA1 *mut*⁺ cells) was labelled twice, with Alexa 555 and with Alexa 647, respectively. Each pair of targets was hybridised on two distinct arrays on the same slide and was compared to RNA from BRCA1 *wt*⁺ cells as indicated in Table 2. Two experimental replicas in dye swap per sample were thus produced in order to avoid dye effect and mutation effect confounding.

2.6. Microarray data acquisition and analysis

Microarray images were acquired by Gene Pix 4000B dual-laser scanner (Axon Instruments, USA) at 5 µm resolution, 100% gain and variable PMT, depending on the needed colour balancing.

Intensity raw data were extracted from TIF images by using the GenePix PRO 6.0 software (Molecular Devices, Sunnyvale, CA, USA) and were analysed by using LIMMA package,¹⁹ an add-in library of Bioconductor (<http://www.bioconductor.org>).

Before performing the statistical analysis, background was subtracted from the raw data by using the LIMMA package 'minimum' method, which sets any null or negative intensity value generated by the classical background subtraction, equal to half the minimum of the positive corrected intensity values for that array.

Data were normalised within arrays by 'LOWESS' method and between arrays by 'Aquantile' method, both from the LIMMA package. B-statistic²⁰ and adjusted *p*-value (adj-*p*)²¹ were utilised to assign statistical significance to each differentially

Table 2 – Microarray experimental design.

Slide #	Array #	Alexa 555	Alexa 647
1	1_1	M1775R	Wild type
1	1_2	Wild type	M1775R
2	2_1	I1766S	Wild type
2	2_2	Wild type	I1766S
3	3_1	A1789T	Wild type
3	3_2	Wild type	A1789T
4	4_1	S1164I	Wild type
4	4_2	Wild type	S1164I
5	5_1	Y179C	Wild type
5	5_2	Wild type	Y179C

expressed gene. Only genes with $B > 0$ and $\text{adj-}p < 0.01$ were considered.

Pathway analyses were performed by Pathway Explorer (<http://pathwayexplorer.genome.tugraz.at/>). The list of differentially expressed genes was also analysed by an accurate investigation of the literature to search for genes and molecular pathways potentially involved in the observed phenotypes. To this aim we used Saccharomyces Genome Database (SGD) (<http://www.yeastgenome.org>), Ensembl (<http://www.ensembl.org>), information Hyperlinked Over Proteins (iHOP) (<http://www.ihop-net.org/UniPub/iHOP/>), Munich Information centre for Protein Sequences (MIPS) (<http://MIPS.gsf.de>) and PubMed Central (<http://www.ncbi.nlm.nih.gov/sites/entrez?db=pubmed>).

2.7. Microarray data validation by real-time PCR

The total RNA samples that were used for microarray experiments were also used for real-time PCR analysis.

Total RNAs were reverse transcribed with random and oligo-dT primers using the QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA, USA).

PCR primers were designed using the Beacon Designer 4.0 software (Premier Biosoft, International, Palo Alto, CA, USA) and were synthesised by Invitrogen (Carlsbad, CA, USA). Primer sequences are listed out in Table 3.

Real-time PCRs were performed in the iCycler iQ instrument (Biorad, Hercules, CA, USA) with the Brilliant[®]SYBR[®] GreenQPCR Master Mix (Stratagene, La Jolla, CA, USA) mixed with the uracil–DNA–glycosylase (Fermentas, M-Medical, Milan, Italy) and fluorescein (Biorad, Hercules, CA, USA).

For each primer pair, we tested the amplification efficiency by using five serial dilutions of cDNA carried out in duplicate: all primer pairs displayed an efficiency between 85% and 100%.

The stability of three housekeeping genes (PGK1, PDA1 and ORC5) was evaluated by using *geNorm* software.²² Only two of them, PGK1 and PDA1, were used to normalise the expression values of the target genes because the third one, ORC5, did not show a M stability parameter value higher than 1.5 (threshold established by *geNorm*).

Each sample was run in triplicate to calculate the standard deviation (SD) for the three experimental replicates. We considered only the experiments with $\text{SD} < 0.4$ for each group of replicates.

The relative expression levels for the target genes in BRCA1 *mut*⁺ cells with respect to BRCA1 *wt*⁺ cells were calculated by

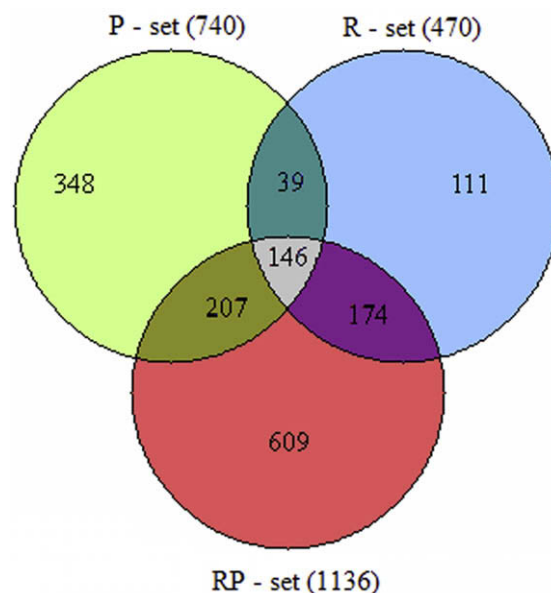


Fig. 1 – Venn diagram of differentially expressed genes by P-, R- and RP-set variants.

the Pfaffl method²³ with the Gene Expression Macro[™] 1.1 application (Bio-Rad, Hercules, CA, USA). They were reported as fold increase or decrease.

3. Results

3.1. Microarray results

Data analysis was performed by grouping the BRCA1 variants (Y179C, S1164I, I1766S, M1775R and A1789T) into three sets: Recombination (R)-set (Y179C and S1164I), Recombination and Proliferation (RP)-set (M1775R and I1766S) and Proliferation (P)-set (A1789T), according to their effects on yeast cell phenotype observed by functional assays,¹³ which are summarised in Table 1.

The analysis revealed 470, 740 and 1136 differentially expressed genes in R-, P- and RP-set, respectively (Fig. 1; Supplementary Tables S1–S3); 353 (207+146) genes were modulated by both P- and RP-mutations ($P \cap RP$), 320 (174+146) by R- and RP-mutations ($R \cap RP$), 185 (39+146) by R- and P-mutations ($R \cap P$) and 146 by R-, RP- and P-mutations ($R \cap RP \cap P$) (Fig. 1). Complete information about the microarray experiments and results can be retrieved from the ArrayExpress

Table 3 – Real-time PCR primer pairs and corresponding genes.

Gene	SGD code	Forward primer	Reverse primer
RNR1	YER070W	5' CGAACCAGTCACTTCCAATATG 3'	5' TCATCCCAATACCTAAATCAACC 3'
POL30	YBR088C	5' ACCCTGTCAATTGCCATCTTC 3'	5' TTAGCTCCGAACGTCAAGTC 3'
SKM1	YOL113W	5' CTGGTCAAGGAGCAAGTGGTG 3'	5' CTACGACTCGGTGGCAATGTG 3'
HHF2	YNL030W	5' GCTAGAAGAGGTGGTGTCAAG 3'	5' GTTCAGTGTAAGTAACAGAGTCC 3'
ADE1	YAR015W	5' CCAAGGCTGAACAAGGTGAAC 3'	5' TTAGTGTCTGCGATGATGATGC 3'
PGK1	YCR012W	5' TCACTCTTCTATGGTCGGTTTCG 3'	5' AATGGTCTGGTTGGGTTCTCC 3'
PDA1	YER178W	5' TTGCTAAGGACTGGTGTCTATC 3'	5' AATCTCGTCTCTAGTTCTGTAGG 3'
ORC5	YNL261W	5' AAGGTAAGCGGAGAGTGG 3'	5' CGTGAATATCGCTGAAGTAATCG 3'

database at the European Bioinformatics Institute (EBI) (<http://www.ebi.ac.uk/arrayexpress/>) by using the following accession number: E-MEXP-1867.

Pathway Explorer mapped about 20% of each set of differentially expressed genes (Supplementary Tables S4–S6).

3.2. Microarray data validation by real-time PCR

Five transcripts among those identified as differentially expressed by microarray analysis were selected for validation by real-time PCR: Ribonucleotide-Diphosphate Reductase, Large Subunit (RNR1), Proliferating Cell Nuclear Antigen (POL30), STE20/PAK Homologous Kinase Related to Morphogenesis (SKM1), Histone H4 protein (HHF2) and N-Succinyl-5-Aminoimidazole-4-Carboxamide Ribonucleotide Synthetase (ADE1), (Table 3). By microarray experiments, RNR1 and POL30 resulted down-regulated in all three sets of variants, SKM1 up-regulated in all three sets and HHF2 and ADE1 down-regulated in R- and RP-set (Fig. 2).

Real-time PCR analysis was performed by considering the variants one by one and the differential expression was consistently confirmed for all the five examined genes (Fig. 2).

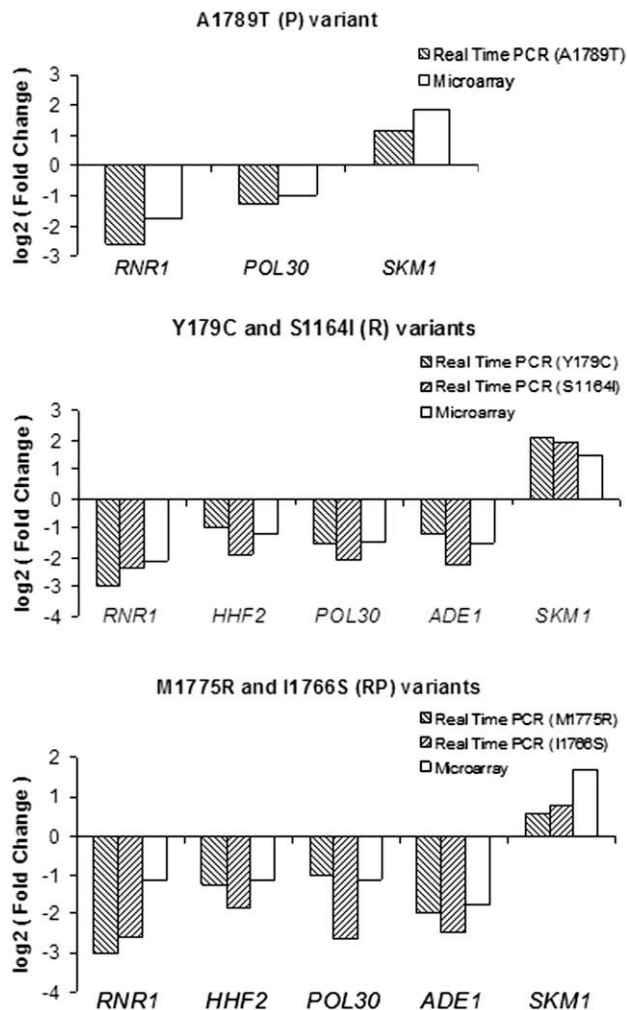


Fig. 2 – Microarray (open bars) and real-time PCR (dashed bars) log-fold changes of genes validated by real-time PCR.

4. Discussion

To investigate the molecular mechanisms that are activated in yeast by potentially deleterious BRCA1 variants, in this study we hybridised the RNA obtained from yeast cells transformed with five variants exhibiting a phenotypic effect either on proliferation and/or on HR on microarrays,¹³ in comparison with the RNA from yeast cells transformed with wild-type BRCA1 (Tables 1 and 2).

Microarray data relative to the five BRCA1 variants were analysed by grouping them into three sets, based on the phenotypes described by Caligo et al.¹³ We assumed that the genes that are consistently induced or repressed by different variants producing the same phenotype would be functionally correlated with the phenotype itself.

That only 20% of the differentially expressed genes were mapped by Pathway Explorer is due to the fact that pathway analysis of yeast genes is still little informative, as a very small number of yeast genes have been placed on KEGG (<http://www.genome.jp/kegg/>) pathways by means of their homology to human genes, and most of them are involved in metabolism. This is why most differentially expressed genes placed by Pathway Explorer were assigned to metabolic pathways, including nucleotide metabolism. Other interesting pathways such as cell cycle, and DNA replication and repair emerged from the analysis, but the small numbers of homologous genes till now assigned to these pathways did not allow for the detection of meaningful differences among the three lists of differentially expressed genes. Thus, the gene lists were analysed by screening the literature and some groups of functionally related genes, which might be more directly involved in the induction of the observed phenotypes, were identified.

4.1. Genes potentially involved in the induction of homologous recombination

Chromatin assembly – HHF2, HTA2 and HTB2, encoding histone proteins H4, H2A and H2B, respectively, were down-regulated by R- and RP-set variants. HTB2 was also down-regulated by the A1789T (P) variant. Interestingly, partial depletion of histone H4 has been shown to induce an increase in HR in yeast.²⁴ H2Ax, the mammalian homologue of H2A, facilitates the assembly of specific DNA repair-complexes on damaged DNA.²⁵

The genes encoding for two components of histone acetyltransferase B, HIF1 and HAT1, turned out to be down-regulated by RP-set variants only and by all three sets, respectively. Acetylation of newly synthesised histone H4 by histone acetyltransferase B plays a role in telomeric silencing and double-strand break (DSB) repair.²⁶

Nucleotide metabolism – An alteration of nucleotide metabolism can contribute to HR as suggested by Yuen et al.,²⁷ who, in a systematic screen for genes associated to chromosome instability, showed that several genes coding for enzymes of the adenosine biosynthetic pathway, when deleted, lead to an increased frequency of chromosomal rearrangements. In the present work, three ADE genes (ADE1, ADE13 and ADE17) turned out to be down-regulated

by R- and RP-set BRCA1 variants. ADE6 was down-regulated by RP-set variants. ADE4 was down-regulated by all three sets of variants.

URA2, which was down-regulated by RP-set variants, and URA3, which was down-regulated by both RP- and P-set variants, are structural genes involved in the *de novo* biosynthesis of uridine monophosphate (UMP).²⁸

DCD1, which was down-regulated by RP-set variants, is a dCMP deaminase required for dCTP and dTTP synthesis.²⁹

PRS4, which was down-regulated by RP-set variants, is a phosphoribosylpyrophosphate synthetase required for nucleotide, histidine and tryptophan biosynthesis.³⁰

DUT1, which was down-regulated by all three sets of variants, encodes a dUTPase: by hydrolysing dUTP to dUMP and PPi, DUT1 prevents incorporation of uracil into DNA during replication, thus participating in the maintenance of genome integrity.³¹

Transcription – SUB2 and RPB8 are down-regulated by R- and RP-set variants. SUB2 protein is a putative RNA helicase which promotes transcriptional elongation and suppresses transcription-associated recombination.³² SUB2 inactivation has been shown to increase the recombination rate 800-fold.³³ RPB8 is a highly conserved subunit present in all three eukaryotic RNA polymerases. Although its function is still unclear, both yeast and human RPB8 proteins can bind single-stranded oligonucleotides,^{34,35} and human RPB8 is polyubiquitinated by BRCA1 in response to DNA damage.³⁶

DNA replication and repair – POL30, whose expression is down-regulated by all three sets of variants, encodes the Proliferative Cell Nuclear Antigen (PCNA), a homotrimeric complex that functions as a sliding clamp and processivity factor for DNA polymerases;³⁷ it is also required for sister chromatid cohesion³⁸ and multiple forms of DNA repair.³⁹ Interestingly, ectopic expression of wild-type BRCA1 in human cell lines up-regulates PCNA, the human homologue of POL30.⁴⁰

The genes coding for three subunits of replication factor C, RFC3, RFC4 and RFC5, were down-regulated by both RP- and P-set variants: replication factor C is a DNA-binding protein and ATPase that acts as a clamp loader of the proliferating cell nuclear antigen (PCNA).⁴¹

PCNA interacts with RAD27 (whose mRNA is down-regulated by all three sets of variants)⁴² and POL32 (whose mRNA is down-regulated by RP-set variants),⁴³ among other proteins. RAD27 is a flap endonuclease which, in cooperation with the product of RNH201 (down-regulated by RP-set variants) removes RNA primers from Okazaki fragments during DNA lagging strand synthesis, a critical process for the maintenance of genome integrity.⁴⁴ POL32 is the third subunit of DNA polymerase delta; pol32delta mutants show severe defects in DNA repair, replication and mutagenesis.⁴⁵

R-set variants up-regulated SOH1, which encodes a protein interacting with factors involved in DNA repair and transcription,⁴⁶ and down-regulated NHP6B, a member of the high-mobility group box (HMGB) superfamily whose loss leads to genomic instability and hypersensitivity to DNA-damaging agents in yeast; mouse fibroblasts lacking NHP6B homologue, HMGB1, display higher rates of DNA damage after UV irradiation and chromosomal instability.⁴⁷

The product of SEM1 (down-regulated by RP-set variants) is one of the components of the regulatory cap of 26S protea-

some, a complex involved in protein degradation and DNA DSB repair.⁴⁸ The human SEM1 homologue, DSS1, interacts with BRCA2⁴⁹ which, in turn, has been shown to interact with BRCA1 at sites of DNA damage.⁵⁰ Given the conserved role of SEM1/DSS1 in HR, its down-regulation might represent a possible mechanism through which some mutant forms of BRCA1 lead to cancer development.

4.2. Genes potentially involved in proliferation recovery

Cell cycle – The expression of several cell cycle genes was altered by the P-set variant. These include CDC6, encoding a component of the pre-replicative complex,⁵¹ some cyclin genes (CLN1 and CLB6)^{52,53} and some genes coding for proteins involved in cell cycle checkpoints (RFC5, DRC1, DDC1 and IPL1),^{54–57} all down-regulated, and the G1 cyclin gene CLN,⁵⁸ which is up-regulated. The transcription of CDC6, DRC1, CLN1, CLB6 and IPL1 is regulated by MBF and SBF transcription complexes,^{57,59} suggesting that BRCA1 might interact, directly or indirectly, with these complexes. Interestingly, in mammalian cells, BRCA1 interacts with hypophosphorylated RB which, in turn, interacts with the E2F transcription factor, a functional homologue of MBF and SBF which coordinates the transcription of cell cycle genes.^{7,9,60}

Nucleotide metabolism – The ribonucleotide reductase small subunit genes RNR2 and RNR4,⁶¹ are up-regulated by RP-set variants. The human homologue of RNR2 promotes malignant progression in mammalian cells and is up-regulated in premalignant breast disease.^{62,63} The gene coding for the large subunit of ribonucleotide reductase, RNR1, was down-regulated by all three sets of BRCA1 variants.⁶⁴ Interestingly, overexpression of mouse RNR1 suppresses the tumorigenicity of *ras*-transformed cells.⁶⁵

Invasive and pseudohyphal growth – A group of genes related to invasive (haploid) and pseudohyphal (diploid) growth was up-regulated by the P-set variant: FLO11, MEP2, GPA2, HMS1 and ASH1. FLO11 encodes a surface mucin, and represents a key gene of both invasive (haploid) and pseudohyphal (diploid) growth pathways;⁶⁶ MEP2, GPA2, HMS1 and ASH1 are all implicated in a number of signal transduction cascades leading to the activation of FLO11 transcription.^{67–69} Consistently, DIG2, a negative regulator of FLO11 transcription,^{70,71} is down-regulated in cells transformed with the P-set variant.

SKM1, coding for a Ste20/PAK (p21-activated-kinase)-like serine/threonine protein kinase presumably involved in the activation of polarised growth,⁷² is up-regulated by all BRCA1 variant sets. Invasive growth and pseudohyphal growth are characterised by polarised growth, and alterations of motility, cell-cell and cell-substrate adhesiveness and substrate invasiveness.⁷³ These morphological changes recall those of cancer cells; indeed, several homologues of yeast genes involved in cytoskeleton remodelling and cell motility are overexpressed in human cancer cells.⁷⁴ The mucin family is conserved among eukaryotes and overexpression of one mucin or more mucins has been observed in many types of cancers,^{75,76} and the expression level of several human mucins correlates with tumour invasiveness.⁷⁵

Human proteins belonging to the PAK family act downstream of RHO-GTPases, like Cdc42 and Rac proteins, and play a role in modulating the actin cytoskeleton.⁷⁷ Overexpression

of RAC proteins is directly correlated with tumour invasiveness and cellular motility in breast cancer.⁷⁸ In addition, in breast cancer cell lines the human protein PAK1 interacts with histone H3⁷⁹ and its overexpression leads to abnormal assembly of the mitotic spindle.⁸⁰

4.3. Genes potentially involved in both induction of homologous recombination and proliferation recovery

Chromatin remodelling – ARP7, ARP9 and SFH1 were down-regulated by RP-set variants. Arp7, Arp9 and Sfh1 are components of the chromatin remodelling complexes SWI/SNF and RSC,^{81,82} which promote transcription elongation,⁸³ take part in DNA DSB repair⁸⁴ and control progression through the cell cycle.⁸⁵ Interestingly, in human cells, there is evidence that BRCA1 controls transcription via a direct interaction with a SWI/SNF-related complex.⁸⁶ Yeast SFH1 is the homologue of the human tumour suppressor gene SNF5.⁸⁷

Cell cycle checkpoints – TOP2 (down-regulated by RP-set variants) encodes a type II topoisomerase which prevents chromosome aberrations by facilitating the separation between replicated sister chromatids and by preventing recombination between rDNA repeats.^{88,89} Both yeast TOP2 and its human homologue are involved in the G2/M decatenation checkpoint, which, in human cells, requires BRCA1.^{8,90}

The protein encoded by MSH2, also conserved in mammals, forms two protein complexes, with either MSH6 or MSH3, which are able to recognise base-base mispairs and single-base insertions/deletions, or larger insertions/deletions, respectively, to initiate the repair process.⁹¹ In human cells, MSH2 takes part in the BRCA1-associated surveillance complex,⁹² and cooperates with BRCA1 in activating the G2-M checkpoint following DNA damage.⁹³ MSH2 was down-regulated by all three sets of variants.

5. Concluding remarks

The present work highlights a number of genes and molecular pathways which are affected in yeast by five BRCA1 missense mutations in comparison with wild-type BRCA1. These data point to some molecular mechanisms which might be responsible for the effects of BRCA1 variants on yeast phenotype: transcriptional elongation, and DNA replication and repair, whose alterations might contribute to the rise in HR rate; cell cycle checkpoint control and growth regulation, whose alterations probably contribute to proliferation recovery; chromatin assembly and remodelling and nucleotide metabolism, as pathways which might be implicated in both phenomena. The alteration of molecular mechanisms critical for the control of cell proliferation and of genome integrity provides further support to the hypothesis of a pathogenic role of the analysed mutations, already suggested by the results of Caligo et al.¹³ It also supports a dual role of BRCA1 in cancer protection, both as a caretaker and a gatekeeper gene.⁹⁴

Finally, our results confirm that yeast, despite the absence of a BRCA1 homologue, represents a valid model system to examine BRCA1 molecular functions, as the molecular pathways activated by BRCA1 variants are conserved in humans.

Thus, information acquired in the yeast model may contribute to understand the molecular events that occur in human cells as a consequence of BRCA1.

Conflict of interest statement

None declared.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at [doi:10.1016/j.ejca.2009.04.025](https://doi.org/10.1016/j.ejca.2009.04.025).

REFERENCES

1. Fackenthal JD, Olopade OI. Breast cancer risk associated with BRCA1 and BRCA2 in diverse populations. *Nat Rev Cancer* 2007;7(12):937–48.
2. Zhang J, Powell SN. The role of the BRCA1 tumor suppressor in DNA double-strand break repair. *Mol Cancer Res* 2005;3(10):531–9.
3. Deng CX, Brodie SG. Roles of BRCA1 and its interacting proteins. *Bioessays* 2000;22(8):728–37.
4. Bhattacharyya A, Ear US, Koller BH, Weichselbaum RR, Bishop DK. The breast cancer susceptibility gene BRCA1 is required for subnuclear assembly of Rad51 and survival following treatment with the DNA cross-linking agent cisplatin. *J Biol Chem* 2000;275(31):23899–903.
5. Xu X, Aprelikova O, Moens P, Deng CX, Furth PA. Impaired meiotic DNA-damage repair and lack of crossing-over during spermatogenesis in BRCA1 full-length isoform deficient mice. *Development* 2003;130(9):2001–12.
6. Zhong Q, Boyer TG, Chen PL, Lee WH. Deficient nonhomologous end-joining activity in cell-free extracts from BRCA1-null fibroblasts. *Cancer Res* 2002;62(14):3966–70.
7. Deng CX. BRCA1: cell cycle checkpoint, genetic instability, DNA damage response and cancer evolution. *Nucleic Acids Res* 2006;34(5):1416–26.
8. Deming PB, Cistulli CA, Zhao H, et al. The human decatenation checkpoint. *Proc Natl Acad Sci USA* 2001;98(21):12044–9.
9. Aprelikova ON, Fang BS, Meissner EG, et al. BRCA1-associated growth arrest is RB-dependent. *Proc Natl Acad Sci USA* 1999;96(21):11866–71.
10. Mullan PB, Quinn JE, Harkin DP. The role of BRCA1 in transcriptional regulation and cell cycle control. *Oncogene* 2006;25(43):5854–63.
11. Humphrey JS, Salim A, Erdos MR, Collins FS, Brody LC, Klausner RD. Human BRCA1 inhibits growth in yeast: potential use in diagnostic testing. *Proc Natl Acad Sci USA* 1997;94(11):5820–5.
12. Skibbens RV, Ringhoff DN, Marzillier J, Cassimeris L, Eastman L. Positional analyses of BRCA1-dependent expression in *Saccharomyces cerevisiae*. *Cell Cycle* 2008;7(24):3928–34.

13. Caligo MA, Bonatti F, Guidugli L, Aretini P, Galli A. A yeast recombination assay to characterize human BRCA1 missense variants of unknown pathological significance. *Hum Mutat* 2009;**30**(1):123–33.
14. Monteiro AN, August A, Hanafusa H. Evidence for a transcriptional activation function of BRCA1 C-terminal region. *Proc Natl Acad Sci USA* 1996;**93**(24):13595–9.
15. Tischkowitz M, Hamel N, Carvalho MA, et al. Pathogenicity of the BRCA1 missense variant M1775K is determined by the disruption of the BRCT phosphopeptide-binding pocket: a multi-modal approach. *Eur J Hum Genet* 2008;**16**(7):820–32.
16. Carvalho MA, Marsillac SM, Karchin R, et al. Determination of cancer risk associated with germ line BRCA1 missense variants by functional analysis. *Cancer Res* 2007;**67**(4):1494–501.
17. Judkins T, Hendrickson BC, Deffenbaugh AM, et al. Application of embryonic lethal or other obvious phenotypes to characterize the clinical significance of genetic variants found in trans with known deleterious mutations. *Cancer Res* 2005;**65**(21):10096–103.
18. Tavtigian SV, Deffenbaugh AM, Yin L, et al. Comprehensive statistical study of 452 BRCA1 missense substitutions with classification of eight recurrent substitutions as neutral. *J Med Genet* 2006;**43**(4):295–305.
19. Smyth G. Limma: linear models for microarray data. In: Gentleman R, Carey V, Dudoit S, Irizarry R, Huber W, editors. *Bioinformatics and computational biology solutions using R and bioconductor*. New York: Springer; 2005. p. 397–420.
20. Lonnstedt I, Speed T. Replicated microarray data. *Stat Sinica* 2002;**12**:31–46.
21. Benjamini Y, Hochberg Y. Controlling the false discovery rate: a practical and powerful approach to multiple testing. *J Roy Stat Soc Ser B (Meth)* 1995;**57**(1):289–300.
22. Vandesompele J, De Preter K, Pattyn F, et al. Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes. *Genome Biol* 2002;**3**(7):78. RESEARCH0034.
23. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. *Nucleic Acids Res* 2001;**29**(9):e45.
24. Prado F, Aguilera A. Partial depletion of histone H4 increases homologous recombination-mediated genetic instability. *Mol Cell Biol* 2005;**25**(4):1526–36.
25. Celeste A, Petersen S, Romanienko PJ, et al. Genomic instability in mice lacking histone H2AX. *Science* 2002;**296**(5569):922–7.
26. Kelly TJ, Qin S, Gottschling DE, Parthun MR. Type B histone acetyltransferase Hat1p participates in telomeric silencing. *Mol Cell Biol* 2000;**20**(19):7051–8.
27. Yuen KW, Warren CD, Chen O, Kwok T, Hieter P, Spencer FA. Systematic genome instability screens in yeast and their potential relevance to cancer. *Proc Natl Acad Sci USA* 2007;**104**(10):3925–30.
28. Nagy M, Le Gouar M, Potier S, Souciet JL, Herve G. The primary structure of the aspartate transcarbamylase region of the URA2 gene product in *Saccharomyces cerevisiae*. Features involved in activity and nuclear localization. *J Biol Chem* 1989;**264**(14):8366–74.
29. McIntosh EM, Haynes RH. Sequence and expression of the dCMP deaminase gene (DCD1) of *Saccharomyces cerevisiae*. *Mol Cell Biol* 1986;**6**(5):1711–21.
30. Hernando Y, Carter AT, Parr A, Hove-Jensen B, Schweizer M. Genetic analysis and enzyme activity suggest the existence of more than one minimal functional unit capable of synthesizing phosphoribosyl pyrophosphate in *Saccharomyces cerevisiae*. *J Biol Chem* 1999;**274**(18):12480–7.
31. Guillet M, Van Der Kemp PA, Boiteux S. DUTPase activity is critical to maintain genetic stability in *Saccharomyces cerevisiae*. *Nucleic Acids Res* 2006;**34**(7):2056–66.
32. Aguilera A. The connection between transcription and genomic instability. *Embo J* 2002;**21**(3):195–201.
33. Fan HY, Merker RJ, Klein HL. High-copy-number expression of Sub2p, a member of the RNA helicase superfamily, suppresses hpr1-mediated genomic instability. *Mol Cell Biol* 2001;**21**(16):5459–70.
34. Kang X, Hu Y, Li Y, et al. Structural, biochemical, and dynamic characterizations of the hRPB8 subunit of human RNA polymerases. *J Biol Chem* 2006;**281**(26):18216–26.
35. Krapp S, Kelly G, Reischl J, Weinzierl RO, Matthews S. Eukaryotic RNA polymerase subunit RPB8 is a new relative of the OB family. *Nat Struct Biol* 1998;**5**(2):110–4.
36. Wu W, Nishikawa H, Hayami R, et al. BRCA1 ubiquitinates RPB8 in response to DNA damage. *Cancer Res* 2007;**67**(3):951–8.
37. Paunesku T, Mittal S, Protic M, et al. Proliferating cell nuclear antigen (PCNA): ringmaster of the genome. *Int J Radiat Biol* 2001;**77**(10):1007–21.
38. Moldovan GL, Pfander B, Jentsch S. PCNA controls establishment of sister chromatid cohesion during S phase. *Mol Cell* 2006;**23**(5):723–32.
39. Watts FZ. Sumoylation of PCNA: wrestling with recombination at stalled replication forks. *DNA Repair (Amst)* 2006;**5**(3):399–403.
40. MacLachlan TK, Somasundaram K, Sgagias M, et al. BRCA1 effects on the cell cycle and the DNA damage response are linked to altered gene expression. *J Biol Chem* 2000;**275**(4):2777–85.
41. Yao N, Coryell L, Zhang D, et al. Replication factor C clamp loader subunit arrangement within the circular pentamer and its attachment points to proliferating cell nuclear antigen. *J Biol Chem* 2003;**278**(50):50744–53.
42. Refsland EW, Livingston DM. Interactions among DNA ligase I, the flap endonuclease and proliferating cell nuclear antigen in the expansion and contraction of CAG repeat tracts in yeast. *Genetics* 2005;**171**(3):923–34.
43. Johansson E, Garg P, Burgers PM. The Pol32 subunit of DNA polymerase delta contains separable domains for processive replication and proliferating cell nuclear antigen (PCNA) binding. *J Biol Chem* 2004;**279**(3):1907–15.
44. Qiu J, Qian Y, Frank P, Wintersberger U, Shen B. *Saccharomyces cerevisiae* RNase H(35) functions in RNA primer removal during lagging-strand DNA synthesis, most efficiently in cooperation with Rad27 nuclease. *Mol Cell Biol* 1999;**19**(12):8361–71.
45. Gerik KJ, Li X, Pautz A, Burgers PM. Characterization of the two small subunits of *Saccharomyces cerevisiae* DNA polymerase delta. *J Biol Chem* 1998;**273**(31):19747–55.
46. Linder T, Gustafsson CM. The Soh1/MED31 protein is an ancient component of *Schizosaccharomyces pombe* and *Saccharomyces cerevisiae* mediator. *J Biol Chem* 2004;**279**(47):49455–9.
47. Giavara S, Kosmidou E, Hande MP, et al. Yeast Nhp6A/B and mammalian Hmgb1 facilitate the maintenance of genome stability. *Curr Biol* 2005;**15**(1):68–72.
48. Krogan NJ, Lam MH, Fillingham J, et al. Proteasome involvement in the repair of DNA double-strand breaks. *Mol Cell* 2004;**16**(6):1027–34.
49. Marston NJ, Richards WJ, Hughes D, Bertwistle D, Marshall CJ, Ashworth A. Interaction between the product of the breast cancer susceptibility gene BRCA2 and DSS1, a protein functionally conserved from yeast to mammals. *Mol Cell Biol* 1999;**19**(7):4633–42.
50. Zhang J, Powell SN. The role of the BRCA1 tumor suppressor in DNA double-strand break repair. *Mol Cancer Res* 2005;**3**(10):531–9.

51. Piatti S, Lengauer C, Nasmyth K. Cdc6 is an unstable protein whose de novo synthesis in G1 is important for the onset of S phase and for preventing a 'reductional' anaphase in the budding yeast *Saccharomyces cerevisiae*. *Embo J* 1995;14(15):3788–99.
52. Schwob E, Nasmyth K. CLB5 and CLB6, a new pair of B cyclins involved in DNA replication in *Saccharomyces cerevisiae*. *Gene Dev* 1993;7(7A):1160–75.
53. Dirick L, Bohm T, Nasmyth K. Roles and regulation of Cln–Cdc28 kinases at the start of the cell cycle of *Saccharomyces cerevisiae*. *Embo J* 1995;14(19):4803–13.
54. Biggins S, Murray AW. The budding yeast protein kinase Ipl1/Aurora allows the absence of tension to activate the spindle checkpoint. *Gene Dev* 2001;15(23):3118–29.
55. Longhese MP, Paciotti V, Fraschini R, Zaccarini R, Plevani P, Lucchini G. The novel DNA damage checkpoint protein ddc1p is phosphorylated periodically during the cell cycle and in response to DNA damage in budding yeast. *Embo J* 1997;16(17):5216–26.
56. Sugimoto K, Shimomura T, Hashimoto K, Araki H, Sugino A, Matsumoto K. Rfc5, a small subunit of replication factor C complex, couples DNA replication and mitosis in budding yeast. *Proc Natl Acad Sci USA* 1996;93(14):7048–52.
57. Wang H, Elledge SJ. DRC1, DNA replication and checkpoint protein 1, functions with DPB11 to control DNA replication and the S-phase checkpoint in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci USA* 1999;96(7):3824–9.
58. Tyers M, Tokiwa G, Fitcher B. Comparison of the *Saccharomyces cerevisiae* G1 cyclins: Cln3 may be an upstream activator of Cln1, Cln2 and other cyclins. *Embo J* 1993;12(5):1955–68.
59. Nasmyth K, Dirick L. The role of SWI4 and SWI6 in the activity of G1 cyclins in yeast. *Cell* 1991;66(5):995–1013.
60. Hateboer G, Wobst A, Petersen BO, et al. Cell cycle-regulated expression of mammalian CDC6 is dependent on E2F. *Mol Cell Biol* 1998;18(11):6679–97.
61. Abid MR, Li Y, Anthony C, De Benedetti A. Translational regulation of ribonucleotide reductase by eukaryotic initiation factor 4E links protein synthesis to the control of DNA replication. *J Biol Chem* 1999;274(50):35991–8.
62. Jensen RA, Page DL, Holt JT. Identification of genes expressed in premalignant breast disease by microscopy-directed cloning. *Proc Natl Acad Sci USA* 1994;91(20):9257–61.
63. Fan H, Villegas C, Wright JA. Ribonucleotide reductase R2 component is a novel malignancy determinant that cooperates with activated oncogenes to determine transformation and malignant potential. *Proc Natl Acad Sci USA* 1996;93(24):14036–40.
64. Nordlund P, Reichard P. Ribonucleotide reductases. *Annu Rev Biochem* 2006;75:681–706.
65. Fan H, Huang A, Villegas C, Wright JA. The R1 component of mammalian ribonucleotide reductase has malignancy-suppressing activity as demonstrated by gene transfer experiments. *Proc Natl Acad Sci USA* 1997;94(24):13181–6.
66. Lambrechts MG, Bauer FF, Marmur J, Pretorius IS. Muc1, a mucin-like protein that is regulated by Mss10, is critical for pseudohyphal differentiation in yeast. *Proc Natl Acad Sci USA* 1996;93(16):8419–24.
67. Lorenz MC, Heitman J. The MEP2 ammonium permease regulates pseudohyphal differentiation in *Saccharomyces cerevisiae*. *Embo J* 1998;17(5):1236–47.
68. Kubler E, Mosch HU, Rupp S, Lisanti MP. Gpa2p, a G-protein alpha-subunit, regulates growth and pseudohyphal development in *Saccharomyces cerevisiae* via a cAMP-dependent mechanism. *J Biol Chem* 1997;272(33):20321–3.
69. Chandarlapaty S, Errede B. Ash1, a daughter cell-specific protein, is required for pseudohyphal growth of *Saccharomyces cerevisiae*. *Mol Cell Biol* 1998;18(5):2884–91.
70. Bardwell L, Cook JG, Zhu-Shimoni JX, Voora D, Thorner J. Differential regulation of transcription: repression by unactivated mitogen-activated protein kinase Kss1 requires the Dig1 and Dig2 proteins. *Proc Natl Acad Sci USA* 1998;95(26):15400–5.
71. Tedford K, Kim S, Sa D, Stevens K, Tyers M. Regulation of the mating pheromone and invasive growth responses in yeast by two MAP kinase substrates. *Curr Biol* 1997;7(4):228–38.
72. Martin H, Mendoza A, Rodriguez-Pachon JM, Molina M, Nombela C. Characterization of SKM1, a *Saccharomyces cerevisiae* gene encoding a novel Ste20/PAK-like protein kinase. *Mol Microbiol* 1997;23(3):431–44.
73. Palecek SP, Parikh AS, Kron SJ. Sensing, signalling and integrating physical processes during *Saccharomyces cerevisiae* invasive and filamentous growth. *Microbiology* 2002;148(Part 4):893–907.
74. Vega FM, Ridley AJ. Rho GTPases in cancer cell biology. *FEBS Lett* 2008;582(14):2093–101.
75. Rakha EA, Boyce RW, Abd El-Rehim D, et al. Expression of mucins (MUC1, MUC2, MUC3, MUC4, MUC5AC and MUC6) and their prognostic significance in human breast cancer. *Modern Pathol* 2005;18(10):1295–304.
76. Carraway 3rd KL, Funes M, Workman HC, Sweeney C. Contribution of membrane mucins to tumor progression through modulation of cellular growth signaling pathways. *Curr Top Dev Biol* 2007;78:1–22.
77. Hofmann C, Shepelev M, Chernoff J. The genetics of PAK. *J Cell Sci* 2004;117(Part 19):4343–54.
78. Baugher PJ, Krishnamoorthy L, Price JE, Dharmawardhane SF. Rac1 and Rac3 isoform activation is involved in the invasive and metastatic phenotype of human breast cancer cells. *Breast Cancer Res* 2005;7(6):R965–74.
79. Li F, Adam L, Vadlamudi RK, et al. P21-activated kinase 1 interacts with and phosphorylates histone H3 in breast cancer cells. *EMBO Rep* 2002;3(8):767–73.
80. Vadlamudi RK, Adam L, Wang RA, et al. Regulatable expression of p21-activated kinase-1 promotes anchorage-independent growth and abnormal organization of mitotic spindles in human epithelial breast cancer cells. *J Biol Chem* 2000;275(46):36238–44.
81. Cao Y, Cairns BR, Kornberg RD, Laurent BC. Sfh1p, a component of a novel chromatin-remodeling complex, is required for cell cycle progression. *Mol Cell Biol* 1997;17(6):3323–34.
82. Cairns BR, Erdjument-Bromage H, Tempst P, Winston F, Kornberg RD. Two actin-related proteins are shared functional components of the chromatin-remodeling complexes RSC and SWI/SNF. *Mol Cell* 1998;2(5):639–51.
83. Carey M, Li B, Workman JL. RSC exploits histone acetylation to abrogate the nucleosomal block to RNA polymerase II elongation. *Mol Cell* 2006;24(3):481–7.
84. Osley MA, Shen X. Altering nucleosomes during DNA double-strand break repair in yeast. *Trends Genet* 2006;22(12):671–7.
85. Neely KE, Workman JL. The complexity of chromatin remodeling and its links to cancer. *Biochim Biophys Acta* 2002;1603(1):19–29.
86. Bochar DA, Wang L, Beniya H, et al. BRCA1 is associated with a human SWI/SNF-related complex: linking chromatin remodeling to breast cancer. *Cell* 2000;102(2):257–65.
87. Versteeg I, Sevenet N, Lange J, et al. Truncating mutations of hSNF5/INI1 in aggressive paediatric cancer. *Nature* 1998;394(6689):203–6.
88. Christman MF, Dietrich FS, Fink GR. Mitotic recombination in the rDNA of *S. cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. *Cell* 1988;55(3):413–25.
89. Holm C, Stearns T, Botstein D. DNA topoisomerase II must act at mitosis to prevent nondisjunction and chromosome breakage. *Mol Cell Biol* 1989;9(1):159–68.

-
90. Andrews CA, Vas AC, Meier B, et al. A mitotic topoisomerase II checkpoint in budding yeast is required for genome stability but acts independently of Pds1/securin. *Gene Dev* 2006;**20**(9):1162–74.
 91. Wang JY, Edelmann W. Mismatch repair proteins as sensors of alkylation DNA damage. *Cancer Cell* 2006;**9**(6):417–8.
 92. Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. *Gene Dev* 2000;**14**:927–39.
 93. Yamane K, Schupp JE, Kinsella TJ. BRCA1 activates a G2-M cell cycle checkpoint following 6-thioguanine-induced DNA mismatch damage. *Cancer Res* 2007;**67**(13):6286–92.
 94. Kinzler KW, Vogelstein B. Cancer-susceptibility genes. Gatekeepers and caretakers. *Nature* 1997;**386**(6627):761–3.