

RESEARCH ARTICLE

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# Effects on human transcriptome of mutated BRCA1 BRCT domain: A microarray study

Caterina Iofrida<sup>1</sup>, Erika Melissari<sup>1</sup>, Veronica Mariotti<sup>1</sup>, Chiara Guglielmi<sup>2</sup>, Lucia Guidugli<sup>3</sup>, Maria Adelaide Caligo<sup>2</sup> and Silvia Pellegrini<sup>1\*</sup>

## Abstract

**Background:** BRCA1 (breast cancer 1, early onset) missense mutations have been detected in familial breast and ovarian cancers, but the role of these variants in cancer predisposition is often difficult to ascertain. In this work, the molecular mechanisms affected in human cells by two BRCA1 missense variants, M1775R and A1789T, both located in the second BRCT (BRCA1 C Terminus) domain, have been investigated. Both these variants were isolated from familial breast cancer patients and the study of their effect on yeast cell transcriptome has previously provided interesting clues to their possible role in the pathogenesis of breast cancer.

**Methods:** We compared by Human Whole Genome Microarrays the expression profiles of HeLa cells transfected with one or the other variant and HeLa cells transfected with BRCA1 wild-type. Microarray data analysis was performed by three comparisons: M1775R versus wild-type (M1775RvsWT-contrast), A1789T versus wild-type (A1789TvsWT-contrast) and the mutated BRCT domain versus wild-type (MutvsWT-contrast), considering the two variants as a single mutation of BRCT domain.

**Results:** 201 differentially expressed genes were found in M1775RvsWT-contrast, 313 in A1789TvsWT-contrast and 173 in MutvsWT-contrast. Most of these genes mapped in pathways deregulated in cancer, such as cell cycle progression and DNA damage response and repair.

**Conclusions:** Our results represent the first molecular evidence of the pathogenetic role of M1775R, already proposed by functional studies, and give support to a similar role for A1789T that we first hypothesized based on the yeast cell experiments. This is in line with the very recently suggested role of BRCT domain as the main effector of BRCA1 tumor suppressor activity.

**Keywords:** Gene expression, Microarray analysis, Missense mutations, BRCA1 gene, DNA damage, DNA repair, Genomic instability, Cell proliferation, Breast neoplasms, Apoptosis

## Background

*BRCA1* is a tumor suppressor gene whose mutations lead to breast and/or ovarian cancer. Human *BRCA1* encodes a full-length protein of 1863 amino acids that is an important player in controlling cell cycle progression. It is involved in DNA damage response signaling network, participating in G1/S, S and G2/M checkpoints. *BRCA1* is required for TP53 phosphorylation mediated by ATM/ATR (ataxia telangiectasia mutated and ataxia telangiectasia and Rad3 related) in response to DNA

damage by ionizing or ultraviolet irradiation [1]. *BRCA1* is also required for the TP53-mediated activation of *CDKN1A* (cyclin-dependent kinase inhibitor 1A) transcription that leads to cell cycle arrest [2]. Both *BRCA1-ATM* and *BRCA1-ATR* interactions produce the phosphorylation of *BRCA1* on specific Ser/Thr residues, required for cell cycle arrest in S and G2 [3]. *BRCA1* is also involved in maintaining the cell genomic integrity. It forms a complex with *RBBP8* (retinoblastoma binding protein 8) and *MRN* (*MRE11A/RAD50/NBN*: meiotic recombination 11 homolog A (*S. cerevisiae*), *RAD50* homolog (*S. cerevisiae*), *nibrin*) that participates in DNA double-strand break repair mediated by homologous recombination [4]. *BRCA1* is furthermore able to act as

\* Correspondence: [silvia.pellegrini@med.unipi.it](mailto:silvia.pellegrini@med.unipi.it)

<sup>1</sup>Department of Experimental Pathology, Medical Biotechnology, Epidemiology and Infectious Diseases, University of Pisa, 56126, Pisa, Italy  
Full list of author information is available at the end of the article

ubiquitin ligase when heterodimerizes with BARD1 (BRCA1 associated RING domain 1) [5]. The most recent hypothesis on BRCA1 concerns a role in maintaining global heterochromatin integrity that might justify its tumor suppressor function [6].

BRCA1 consists of different functional domains: a N-terminal RING finger domain, two nuclear localization signals, a "SQ" cluster, a branched DNA-binding domain and a C-terminal domain containing two BRCT (BRCA1 C Terminus) repeats [7]. BRCT repeats have been found in many other proteins that regulate DNA damage response and have a crucial role for their function [8]. BRCT repeats have been also described as phosphopeptide-interacting motifs, facilitating the assembly of DNA damage signaling complexes following checkpoint kinases activation [9]. BRCT domains are also involved in the transcriptional activity of BRCA1 and the second BRCT repeat (aa 1760–1863) is critical for the activation of the *CDKN1A* promoter [2]. Finally, a recent paper reported that BRCA1 tumor suppression depends on BRCT phosphoprotein binding [10].

Due to the relevance of this region for BRCA1 function, the study of mutations located in the BRCT domain appears of particular interest.

Aim of this work was to investigate the effects on human cell transcriptome of two missense variants, M1775R and A1789T, both located within the second BRCA1 BRCT domain and isolated from familial breast cancers. In a previous work we examined the expression profiles induced by these two mutations in yeast cells [11]. In a recent paper from Guidugli et al. [12] these two variants were tested in a homologous recombination and a non-homologous end-joining assay in HeLa cells. The A1789T variant significantly altered the non-homologous end-joining activity as compared to BRCA1 wild-type.

Here, we compared the expression profiles of HeLa cells transfected with one or the other *BRCA1* variant with that of HeLa cells transfected with *BRCA1* wild-type. We found alterations of molecular mechanisms critical for cell proliferation control and genome integrity, suggestive of a putative role of these two variants in breast cancer pathogenesis.

## Methods

### BRCA1 missense variants

Both *BRCA1* variants are located within the second BRCT domain and, while M1775R has been widely described as deleterious [13], A1789T has been studied only by our group. In yeast cells both these mutations reverted the growth suppression (small colony) phenotype, but only M1775R induced homologous recombination [14]. In HeLa cells A1789T significantly altered the non-homologous end-joining activity as compared to BRCA1 wild-type [12].

### HeLa cells transfection

Five aliquots of the same clone of HeLa G1 cells were transiently transfected with the pcDNA3-BRCA1 wild-type (wt) vector, five with the pcDNA3-BRCA1-M1775R derivative vector and five with the pcDNA3-BRCA1-A1789T derivative vector as described by Guidugli et al. [12].

Twenty-four hours after transfection, cells were washed twice in PBS 1X, pelleted and immediately used to extract RNA or proteins. The increased expression of BRCA1 was assessed by Western Blot as showed by Guidugli et al. [12].

### Microarray

Gene expression was investigated by Whole Human Genome Oligo Microarrays G4112F (Agilent Technologies, Palo Alto, CA, USA). A reference design was adopted using as reference a pool of all the RNA samples from wild-type clones.

Total RNA was extracted and DNase purified with PerfectPure RNA Cultured Cell Kit (5 PRIME) (Eppendorf, Hamburg, Germany). All RNAs, measured by NanoDrop ND-1000 Spectrophotometer (NanoDrop Technologies, Inc. Wilmington, Del, USA), displayed a 260/280 OD ratio > 1.9. The RNA integrity was verified by 1.2% agarose-formaldehyde gel electrophoresis.

Total RNA samples were amplified and labelled with Quick-Amp Labeling kit (Agilent Technologies, Palo Alto, CA, USA). One hundred  $\mu$ l of In Situ Hybridisation Kit Plus mix (Agilent Technologies, Palo Alto, CA, USA) containing 825 ng of Cy3-labelled aRNA (ranging from 11 to 14 Cy3 pmoles) and 825 ng of Cy5-labelled aRNA (18 Cy5 pmoles) were hybridized to each array at 65 °C for 17 h under constant rotation. The arrays were then washed 1 min at RT in 6X SSPE, 0.005% TritonX-102; 1 min at 37 °C in 0.06X SSPE, 0.005% Triton X-102; 30 sec at RT in Acetonitrile solution (Agilent Technologies, Palo Alto, CA, USA) and 30 sec at RT in Stabilization and Drying solution (Agilent Technologies, Palo Alto, CA, USA).

Microarray images were acquired by the Agilent scanner G2565BA and intensity raw data were extracted by the software Feature Extraction V10.5 (Agilent Technologies, Palo Alto, CA, USA). Data preprocessing and statistical analysis were performed by LIMMA (Linear Model of Microarray Analysis) [15] tool. The intensity raw data were background-subtracted and normalized within-arrays and between-arrays.

The contrast matrix was set to evaluate three comparisons: M1775R vs WT, A1789T vs WT and Mut vs WT, considering the two variants as a whole in the latter case. Statistical significance to each gene in each comparison was assigned by B-statistic [16] and only genes with B-statistic > 0 were included.

The pathway analysis was done by Pathway-Express [17,18]. The identification of the Gene Ontology terms that are significantly over- or under-expressed in the lists of differentially expressed genes (DEGs) was performed with Onto-Express using an hypergeometric statistical model [19,20]. The network of biological interactions among DEGs and relevant biological terms was observed by Coremine [21].

#### RT-qPCR

RT-qPCR was performed by the iCycler iQ instrument (Biorad, Hercules, CA, USA) and the iQ SYBR Green Supermix (Biorad, Hercules, CA, USA). Total RNAs were reverse transcribed by QuantiTect Reverse Transcription kit (Qiagen, Valencia, CA, USA). PCR primers (listed in Table 1) were designed by Beacon Designer 4.0 (Premier Biosoft International, Palo Alto, CA, USA). RT-

qPCR experiments were performed according to MIQE guidelines [22].

Four housekeeping genes (see Table 1), tested for stability by geNorm [23], were used to normalize the differential expression of target genes. The analysis was performed considering the variants separately for the M1775R $\nu$ sWT- and the A1789T $\nu$ sWT- contrasts, but as a whole for the Mut $\nu$ sWT-contrast. One-tailed Wilcoxon signed rank test was applied to evaluate the statistical significance of results adopting a threshold of 0.05.

#### Western blot

Western Blot was performed as previously reported [12].

The level of protein expression was analyzed for: GPR56 (anti-GPR56 rabbit polyclonal antibody H-93: sc-99089, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, dilution 1:1000), MRE11A (anti-MRE11A mouse

**Table 1 Primer sequences**

Gene Symbol	Gene Name	Primer Sequences
Housekeeping genes		
<i>ACTB</i>	actin, beta	F: 5'-AACTGGAACGGTGAAGGTGAC-3' R: 5'-GACTTCCTGTAACAACGCATCTC-3'
<i>HPRT1</i>	hypoxanthine phosphoribosyltransferase 1	F: 5'-ACATCTGGAGTCCTATTGACATCG-3' R: 5'-TTAAACAACAATCCGCCAAAGG-3'
<i>GAPDH</i>	glyceraldehyde-3-phosphate dehydrogenase	F: 5'-GTGAAGGTCCGAGTCAACG-3' R: 5'-GGTGAAGACGCCAGTGGACTC-3'
<i>TBP</i>	TATA box binding protein	F: 5'-GGTGTGTGAGAAGATGGATGTTG-3' R: 5'-CCAGATAGCAGCACGGTATGAG-3'
Target genes		
<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	F: 5'-ACTAGGCGGTTGAATGAGAGGTTTC-3' R: 5'-CAGGTCTGAGTGTCCAGGAAAGG-3'
<i>EDN1</i>	endothelin 1	F: 5'-CCAACCATCTTCACTGGCTTCC-3' R: 5'-GTCAGACACAACACTCCCTTAGG-3'
<i>EEF1E1</i>	eukaryotic translation elongation factor 1 epsilon 1	F: 5'-TGCGGGAGGTTCTTGTCTG-3' R: 5'-CTGTAGACTTGGACCATTGTTTG-3'
<i>GPR56</i>	G protein-coupled receptor 56	F: 5'-CTACAGCCGAAGAATGTGACTC-3' R: 5'-GCAGAAGCAGGATGTTTGGG-3'
<i>MRE11A</i>	MRE11 meiotic recombination 11 homolog A (S. cerevisiae)	F: 5'-GATGATGAAGTCCGTGAGGCTATG-3' R: 5'-TGTTGTTGCTGCTGAGATGC-3'
<i>NFKB1</i>	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	F: 5'-CCGTTGGAATGGTGGAGGTC-3' R: 5'-TTGAGAATGAAGGTGGATGATTGC-3'
<i>OBFC2B</i>	oligonucleotide/oligosaccharide-binding fold containing 2B	F: 5'-GACGATGTTGCAATCTG-3' R: 5'-TGGTCACTGAAGTTAGG-3'
<i>PML</i>	promyelocytic leukemia	F: 5'-CCAAGGCAGTCTCACCCAC-3' R: 5'-TTCGGCATCTGAGTCTTCC-3'
<i>SOD2</i>	superoxide dismutase 2, mitochondrial	F: 5'-GGTGTCCAAGGCTCAGGTTG-3' R: 5'-GTGCTCCACACATCAATCCC-3'

monoclonal antibody 18: sc-135992, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, dilution 1:500); NFκB1 (anti-NFκB1 mouse monoclonal antibody E-10: sc-8414, Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA, dilution 1:100) and PML (anti-PML mouse monoclonal IgG2b clone 36.1-104, Upstate Biotechnology, Inc., Waltham, MA, USA, dilution 1: 500).

## Results

### Microarray results

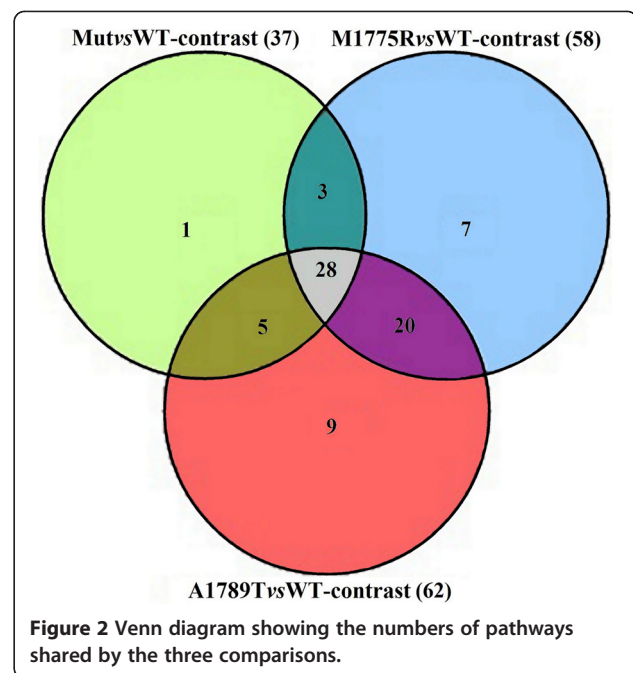
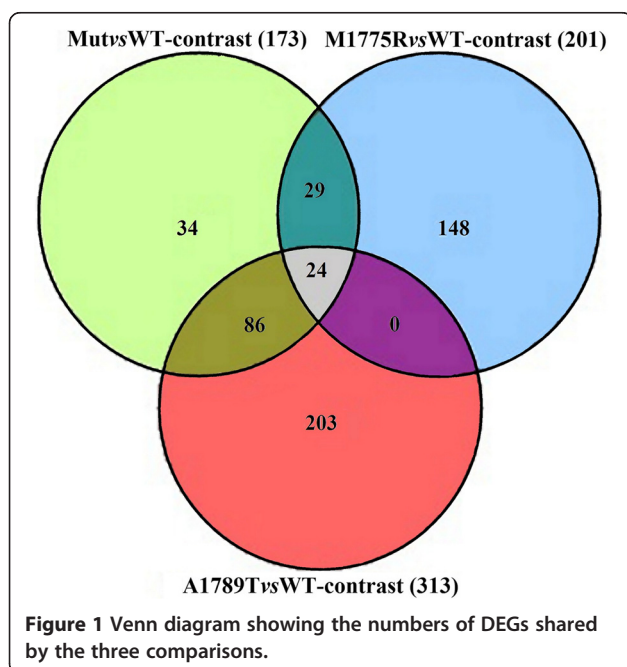
MutvsWT-contrast showed 173 DEGs (Additional file 1), M1775RvsWT-contrast 201 DEGs (Additional file 2) and A1789TvsWT-contrast 313 DEGs (Additional file 3). Twenty-four of these genes were differentially expressed with similar fold changes in all the three comparisons (Figure 1) (Additional file 4).

Complete information about the microarray experiments and results can be retrieved from the ArrayExpress database at the European Bioinformatics Institute [24] by the following accession number: E-MTAB-761.

Pathway analysis mapped 27 DEGs in 37 KEGG pathways for MutvsWT (Additional file 1), 40 DEGs in 58 KEGG pathways for M1775RvsWT (Additional file 2) and 52 DEGs in 62 KEGG pathways for A1789TvsWT (Additional file 3). In all the three comparisons many pathways with high impact factor were involved in cancer.

Twenty-eight pathways were in common among the three comparisons as indicated in Figure 2 (Additional file 5).

Coremine identified 3594 and 2045 genes linked to biological terms concerning “Cell Proliferation” and “DNA damage and repair” processes, respectively



(Additional files 6 and 7). Intersections among these two lists and the three lists of DEGs are shown in Additional files 6 and 7.

### Microarray data validation

The differential expression of nine transcripts (Table 1) identified by microarray analysis was validated by RT-qPCR and consistently confirmed for all the thirteen validations (six for M1775RvsWT, four for A1789TvsWT, three for MutvsWT) (Figure 3).

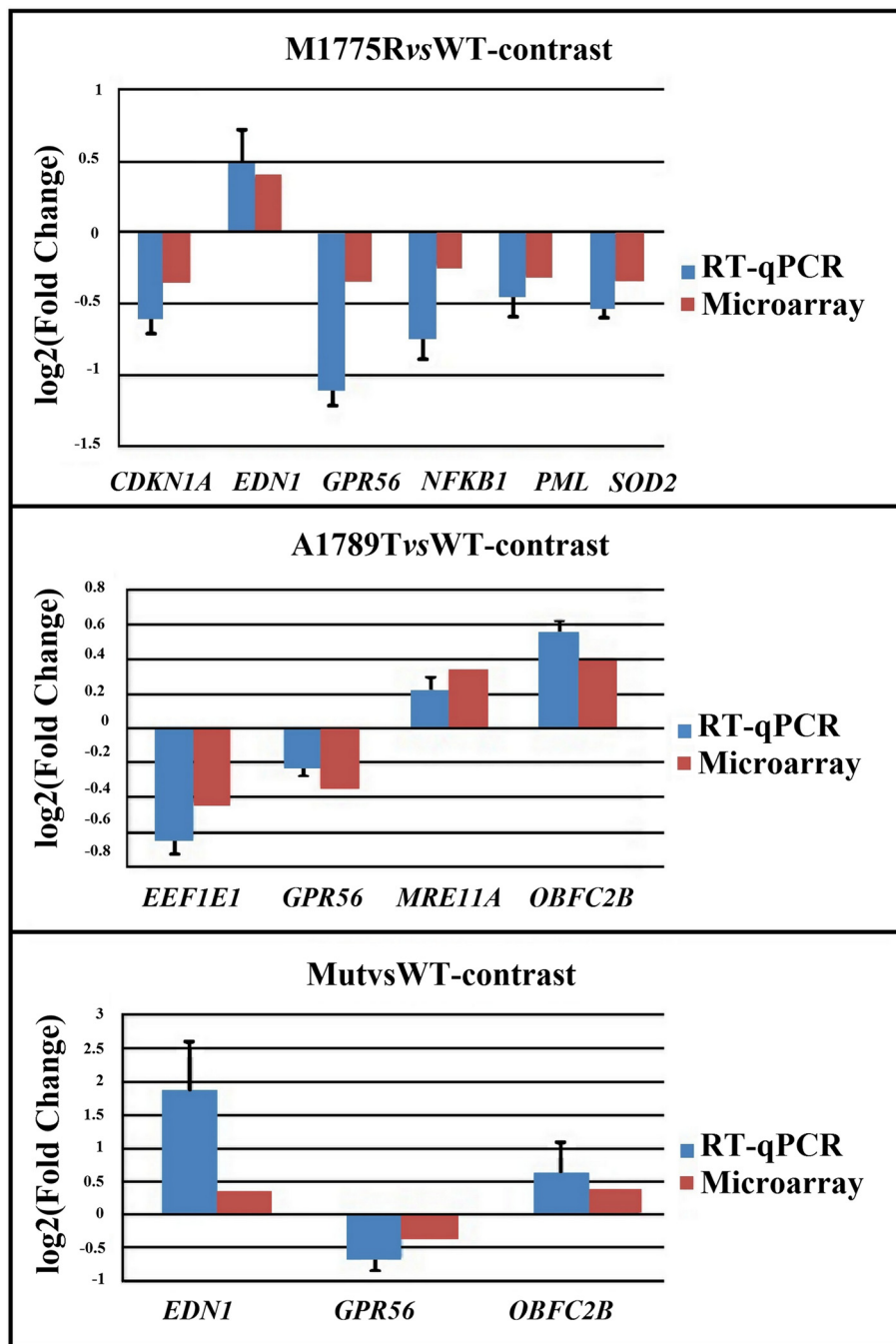
The differential expression of GPR56, MRE11A, PML and NFκB1 proteins was also confirmed by Western Blot analysis (Figure 4).

## Discussion

Aim of this study was the analysis of the effects on human cell transcriptome of two missense variants located in the second BRCT domain of BRCA1, M1775R and A1789T. Specifically, the gene expression profiles of HeLa cells transfected with one or the other variant were compared with that of HeLa cells transfected with BRCA1 wild-type. Three different statistical contrasts were performed: M1775RvsWT, A1789TvsWT and MutvsWT, considering the two variants as a single mutation in the latter case.

Pathway analysis retrieved many pathways involved in cancer onset and progression as well as linked to specific tumors, as shown in Figure 5.

The information retrieved by pathway analysis was completed by ontological and data-mining analyses, which highlighted three functional categories: cell cycle regulation, apoptosis and DNA damage response and

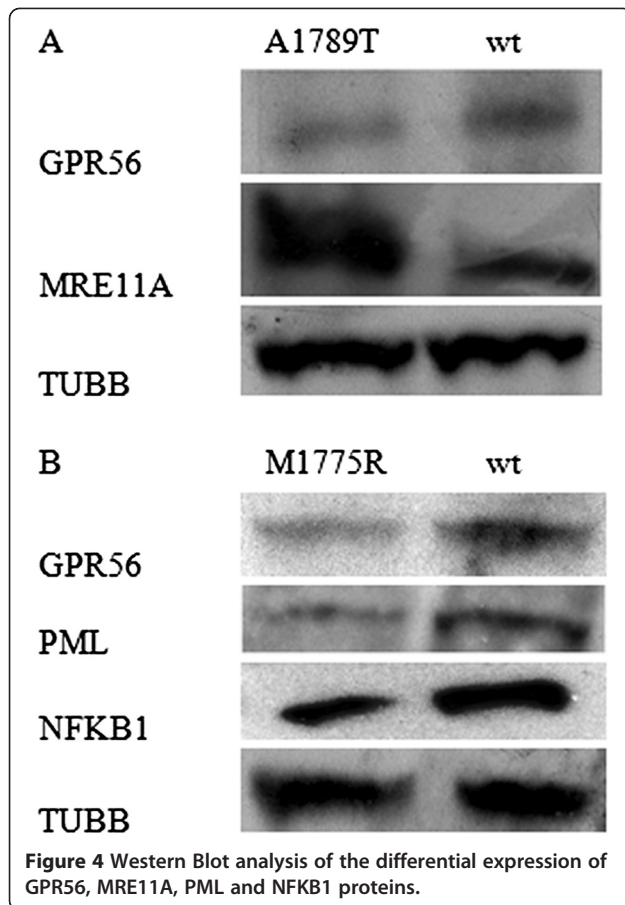


**Figure 3** Microarray and RT-qPCR log<sub>2</sub>-Fold changes for the nine validated genes. All the log<sub>2</sub>-Fold changes are statistically significant (p-value < 0.05).

repair, typically deregulated in cancer cells. Cell cycle and apoptosis deregulation leads to aberrant cell proliferation, while an impaired DNA damage response and repair is known to cause genomic instability. All these processes are closely connected, as apoptosis, constituting a defense from anomalous proliferation, is linked to cell cycle block and is activated in response to DNA damage.

#### Aberrant cell proliferation

Cancer cells proliferate abnormally. In these cells, the mechanisms ensuring correct cell division, which involve cell cycle arrest at checkpoints, are impaired and there is overexpression of mitogenic factors, such as cell cycle positive regulators. Moreover, in cancer cells apoptosis is often downregulated [25-27].



In our data, a considerable number of differentially expressed genes is strictly linked to cell proliferation.

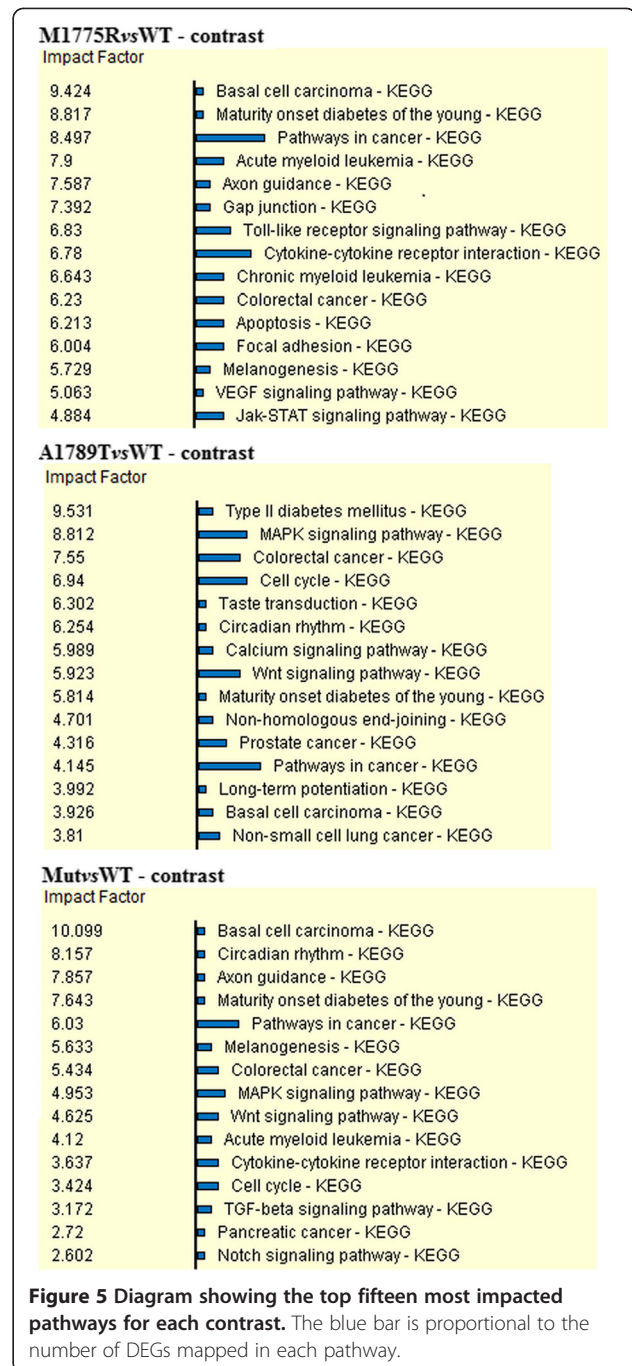
The DEGs linked to cell proliferation were involved in three main phenomena: cell cycle arrest impairment, cell proliferation enhancement and apoptosis blocking (Table 2).

#### Cell cycle arrest impairment

CDKN1A, downregulated by M1775R, is a main effector of cell cycle arrest in response to DNA damage and a promoter of apoptosis [28]. Its expression is usually activated by BRCA1 [2].

Cell cycle can be also arrested by the cooperation of CDKN1A with CEBPA that was in turn downregulated by M1775R [29].

CDKN1A expression is normally activated also by SMAD3, a known transcription factor that acts as an effector of the TGF-beta pathway [30], downregulated in all the three comparisons. The overexpression of SMAD3 in a breast cancer cell line has been shown to cause cell cycle arrest [31], while in SMAD3<sup>-/-</sup> mammary epithelial cells, both TGF-beta-induced growth inhibition and apoptosis are lost [32].



SMAD3 also contributes to the 3-indole-induced G1 arrest in cancer cells [33] and its inhibition depends on CCND1-CDK4 (cyclin-dependent kinase 4) action in breast cancer cells overexpressing CCND1 [34], which appeared upregulated by A1789T. The loss or reduction of BRCA1 expression, moreover, significantly reduces the TGF-beta induced activation of SMAD3 in breast cancer cells [35].

Four other genes linked to cell cycle control appeared downregulated, two, PML and RUVBL1, by M1775R and

**Table 2 Genes linked to aberrant cell proliferation**

Biological Process	Gene Symbol	Gene Name	Contrast	log2 (Fold Change)
Cell cycle arrest impairment	<i>CDKN1A</i>	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	M1775RvsWT	-0.3066647
	<i>CEBPA</i>	CCAAT/enhancer binding protein (C/EBP), alpha	M1775RvsWTMutvsWT	-0.3728651
				-0.3190284
	<i>SMAD3</i>	SMAD family member 3	A1789TvsWTM1775RvsWTMutvsWT	-0.2675322
				-0.4286813
				-0.3196246
	<i>CCND1</i>	cyclin D1	A1789TvsWT	0.3622112
	<i>PML</i>	promyelocytic leukemia	M1775RvsWT	-0.3045759
	<i>RUVBL1</i>	RuvB-like 1 (E. coli)	M1775RvsWT	-0.3028029
	<i>TXNIP</i>	thioredoxin interacting protein	A1789TvsWT	-0.3985633
	<i>RASSF1</i>	Ras association (RalGDS/AF-6) domain family member 1	A1789TvsWT	-0.2766158
Cell proliferation enhancement	<i>FOS</i>	FBJ murine osteosarcoma viral oncogene homolog	A1789TvsWTM1775RvsWTMutvsWT	0.4515777
				0.4020256
				0.4365775
	<i>DUSP1</i>	dual specificity phosphatase 1	A1789TvsWTM1775RvsWTMutvsWT	0.3844494
				0.7606655
				0.5060076
	<i>DUSP2</i>	dual specificity phosphatase 2	MutvsWT	0.5408689
	<i>EDN1</i>	endothelin 1	M1775RvsWTMutvsWT	0.4442705
				0.3212824
	<i>SKP1</i>	S-phase kinase-associated protein 1	A1789TvsWT	0.3353208
<i>ZWILCH</i>	Zwilch, kinetochore associated, homolog (Drosophila)	A1789TvsWT	0.2508541	
	<i>GPR56</i>	G protein-coupled receptor 56	A1789TvsWTM1775RvsWTMutvsWT	-0.3453577
			-0.3310188	
			-0.3407359	
Apoptosis blocking	<i>NFKB1</i>	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	M1775RvsWT	-0.2522979
	<i>TNFRSF10B</i>	tumor necrosis factor receptor superfamily, member 10b	M1775RvsWT	-0.247568
	<i>DYRK2</i>	dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	M1775RvsWT	-0.282513
	<i>PLEKHF1</i>	pleckstrin homology domain containing, family F (with FYVE domain) member 1	MutvsWT	-0.2374774

two, *TXNIP* and *RASSF1*, by A1789T. *PML* codifies for a phosphoprotein localized in nuclear bodies involved in recognition and/or processing of DNA breaks and able to arrest cell cycle in G1 by recruiting TP53 and MRE11A [36]; *RUVBL1* encodes a highly conserved ATP-dependent DNA helicase that plays a role in apoptosis and DNA repair [37]; *TXNIP* acts as a tumor suppressor, as its transfection induces cell-cycle arrest in G0/G1 and is downregulated in human tumors [38] and *RASSF1* is a tumor suppressor that blocks cell cycle progression by inhibiting *CCND1* accumulation. It is

epigenetically inactivated in many tumors, including breast cancer [39,40].

#### Cell proliferation enhancement

The transcription factor *FOS*, upregulated in all the three comparisons, is a well known protooncogene that positively regulates cell cycle progression [41] and is induced in human breast cancer cell cultures [25].

*DUSP1*, upregulated in all the three comparisons, and *DUSP2*, upregulated in MutvsWT, belong to a subfamily of tyrosine phosphatases that regulate the activity of

Mitogen-Activated Protein Kinases (MAPKs). MAPKs are key effectors for cell growth control and survival in physiological and pathological conditions, including cancer and DUSPs have been therefore proposed as potential targets for anticancer drugs [42]. DUSP1 inhibits apoptosis in human mammary epithelial and breast carcinoma cells [43] and its expression is upregulated in many breast cancers [44]. The overexpression of DUSP2 in ovarian cancers has been correlated with poor outcome [45].

EDN1, upregulated by M1775R and in MutvsWT, is a vasoconstrictor that has also co-mitogenic activity, potentiating the growth factor effects. Altered EDN1 signalling is involved in carcinogenesis by modulating cell survival and promoting invasiveness [46].

SKP1, upregulated by A1789T, is a component of the SCF complex that mediates the ubiquitination of cell cycle proteins promoting cell cycle progression [47].

ZWILCH, upregulated by A1789T, is an essential component of the mitotic checkpoint that prevents cells from exiting mitosis prematurely [48].

GPR56, downregulated in all the three contrasts, is a G protein-coupled receptor involved in adhesion processes that participates in cytoskeletal signaling, cellular adhesion and tumor invasion. It is downregulated in melanoma cell lines, while its overexpression suppresses tumor growth and metastasis [49].

#### Apoptosis blocking

NFKB1, downregulated by M1775R, is a pleiotropic transcription factor involved in many biological processes

like inflammation, immunity, differentiation, cell growth, tumorigenesis and apoptosis. Whether NFKB activation contributes or not to cancer is controversial [50], as it regulates the expression of both antiapoptotic [51] and proapoptotic genes [52,53].

Interestingly, *TNFRSF10B*, that was in turn downregulated by M1775R, is one of the proapoptotic genes upregulated by NFKB [53]. TNFRSF10B is one of the two apoptosis-activating receptors binding TNFSF10 (tumor necrosis factor (ligand) superfamily, member 10) [54] that, together with FADD (Fas(TNFRSF6)-Associated via Death Domain) forms a complex that leads to apoptosis through caspases activation [55].

DYRK2, downregulated by M1775R, is a protein kinase that regulates TP53 in inducing apoptosis in response to DNA damage [56] and PLEKHF1, downregulated in MutvsWT, is a recently discovered lysosome-associated protein that activates apoptosis [57] by interacting with the TP53 transactivation domain [58].

#### Genomic instability

An improper reaction to genotoxic stress causes genomic instability, leading to tumorigenesis. Deficiencies in DNA damage signaling and repair pathways are thus fundamental to the etiology of cancer [59].

Among the DEGs involved in genotoxic stress response, some were downregulated causing an increase in genomic instability, others were upregulated (Table 3). Many tumors, including BRCA1-deficient breast cancers, show an overexpression of genes linked to DNA repair that correlates with chemoresistance and poor prognosis [60,61].

**Table 3 Genes linked to genomic instability**

Biological Process	Gene Symbol	Gene Name	Contrast	log2 (Fold Change)
DNA damage response and repair downregulation	<i>EEF1E1</i>	eukaryotic translation elongation factor 1 epsilon 1	A1789TvsWT	-0.4309041
	<i>SMC1A</i>	structural maintenance of chromosomes 1A	A1789TvsWT MutvsWT	-0.2754507-0.2640263
	<i>PPP1CC</i>	protein phosphatase 1, catalytic subunit, gamma isozyme	A1789TvsWT	-0.4286825
	<i>AHNAK</i>	AHNAK nucleoprotein	A1789TvsWT M1775RvsWT MutvsWT	-0.3988113 -0.3103867 -0.3940570
	<i>SOD2</i>	superoxide dismutase 2, mitochondrial	M1775RvsWT MutvsWT	-0.3376169 -0.2502831
DNA damage response and repair upregulation	<i>MRE11A</i>	MRE11 meiotic recombination 11 homolog A ( <i>S. cerevisiae</i> )	A1789TvsWT	0.3293561
	<i>TERF1</i>	telomeric repeat binding factor (NIMA-interacting) 1	MutvsWT	0.2790907
	<i>OBFC2A</i>	oligonucleotide/oligosaccharide-binding fold containing 2A	M1775RvsWT	0.3666172
	<i>OBFC2B</i>	oligonucleotide/oligosaccharide-binding fold containing 2B	A1789TvsWT MutvsWT	0.4070777 0.3417360



Moreover, an increased nuclear staining of DNA repair proteins has been recently observed in tissue sections of breast cancers carrying the M1775R mutation, suggesting a new mechanism of tumorigenesis that involves an enhance of homologous recombination [62].

#### **DNA damage response and repair downregulation**

EEF1E1, downregulated by A1789T, first discovered as associated with a macromolecular tRNA synthetase complex, is a key factor for ATM/ATR-mediated TP53 activation in response to DNA damage [63].

*SMC1A*, downregulated by A1789T and in *MutvsWT*, encodes an evolutionarily conserved chromosomal protein, component of the cohesin complex [64]. *SMC1A* associates with *BRCA1* and is phosphorylated in response to ionizing radiations in an ATM- and NBN-dependent manner [65].

PPP1CC, downregulated by A1789T, is the catalytic subunit of the gamma isoform of PP1 which is a component of a signaling complex, PPP1R1A/PPP1R15A/PP1 that positively regulates apoptosis in response to various stresses, including growth arrest and DNA damage [66].

*AHNAK*, downregulated in all the three contrasts, encodes a protein typically repressed in human neuroblastoma cell lines and in other types of tumors [67]. It firmly binds the LIG4-XRCC4 (ligase IV, DNA, ATP-dependent and X-ray repair complementing defective repair in Chinese hamster cells 4) complex on DNA stimulating its double-stranded ligation activity [68].

*SOD2*, downregulated by M1775R and in *MutvsWT*, is a member of the iron/manganese superoxide dismutase family that acts as a free radical scavenger. It is a candidate tumor suppressor gene as the loss of heterozygosity of its region on chromosome 6 has been found in about 40% of human malignant melanomas [69] and the deletion of chromosome 6 long arm has been identified in SV40 transformed human fibroblasts [70]. In addition, *SOD2* overexpression suppresses the tumorigenicity of breast cancer cells [71].

#### **DNA damage response and repair upregulation**

*MRE11A*, upregulated by A1789T, encodes a component of BASC (Brca1 Associated genome Surveillance Complex), which specifically promotes non-homologous end-joining [72,73]. Interestingly, the A1789T variant altered the non-homologous end-joining activity in a functional assay [11].

*TERF1*, upregulated in *MutvsWT*, is a telomere-associated protein, member of the telomere nucleoprotein complex that interacts with various polypeptides, like the MRN complex [74].

*OBFC2A*, upregulated by M1775R, and *OBFC2B*, upregulated by A1789T and in *MutvsWT*, encode single-stranded DNA-binding proteins essential for DNA

replication, recombination and damage detection and repair. *OBFC2B*, in particular, as an early participant in DNA damage response, is critical for genomic stability [75].

## **Conclusions**

As we first observed in yeast cells [11], also in human cells the *BRCA1* variants M1775R and A1789T affect the expression of many genes critical for cell proliferation and genome integrity maintenance. Our results represent the first molecular confirmation of the pathogenetic role of M1775R. In fact, although more than an evidence exists on the pathogenetic role of this *BRCA1* variant, the effect of this mutation on human cell transcriptome has never been investigated before.

Concerning the A1789T variant, it has been studied only by our group. On the basis of experiments in yeast, we previously suggested for this mutation a causative role in breast cancer onset and development similar to that of M1775R. The present work gives further support to this hypothesis.

## **Additional files**

**Additional file 1: Microarray results of *MutvsWT*-contrast.** The four tabs contain the DEGs, the pathway analysis results and the mapped genes by Pathway-Express and the ontological analysis results by Onto-Express, respectively.

**Additional file 2: Microarray results of M1775RvsWT-contrast.** The four tabs contain the DEGs, the pathway analysis results and the mapped genes by Pathway-Express and the ontological analysis results by Onto-Express, respectively.

**Additional file 3: Microarray results of A1789TvsWT-contrast.** The four tabs contain the DEGs, the pathway analysis results and the mapped genes by Pathway-Express and the ontological analysis results by Onto-Express, respectively.

**Additional file 4: Intersections among the three lists of DEGs.**

**Additional file 5: Intersections among the three lists of pathways.**

**Additional file 6: Intersections among the three lists of DEGs and the list of genes related to "Cell Proliferation" biological term by Coremine.**

**Additional file 7: Intersections among the three lists of DEGs and the list of genes related to "DNA damage and repair" biological term by Coremine.**

## **Competing interests**

The authors declare that they have no competing interests.

## **Authors' contributions**

CI contributed to conceive the study, carried out the RT-qPCR experiments, performed the biological interpretation of microarray data and drafted the manuscript. EM conceived the experimental design, performed the statistical analysis and contributed to draft the manuscript. VM carried out the microarray experiments and contributed to draft the manuscript. CG carried out the western blot experiments and contributed to the biological interpretation of microarray data. LG performed the cell transfection. MAC contributed to conceive the study and to the writing up of the manuscript. SP conceived the study, supervised the experiments, contributed to the interpretation of the results and to the writing up of the manuscript. All authors read and approved the final version of the manuscript.

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## Author details

<sup>1</sup>Department of Experimental Pathology, Medical Biotechnology, Epidemiology and Infectious Diseases, University of Pisa, 56126, Pisa, Italy. <sup>2</sup>Section of Genetic Oncology Division of Surgical, Molecular and Ultrastructural Pathology, Department of Oncology, University of Pisa and Pisa University Hospital, 56126, Pisa, Italy. <sup>3</sup>Laboratory of Medicine and Pathology, Mayo Clinic, Rochester, MN, USA.

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## References

- Fabbro M, Savage K, Hobson K, Deans AJ, Powell SN, McArthur GA, Khanna KK: **BRCA1-BARD1 complexes are required for p53Ser-15 phosphorylation and a G1/S arrest following ionizing radiation-induced DNA damage.** *J Biol Chem* 2004, **279**:31251–31258.
- Chai YL, Cui J, Shao N, Shyam E, Reddy P, Rao VN: **The second BRCT domain of BRCA1 proteins interacts with p53 and stimulates transcription from the p21WAF1/CIP1 promoter.** *Oncogene* 1999, **18**:263–268.
- Ouchi T: **BRCA1 phosphorylation: biological consequences.** *Cancer Biol Ther* 2006, **5**:470–475.
- Chen L, Nievera CJ, Lee AY, Wu X: **Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair.** *J Biol Chem* 2008, **283**:7713–7720.
- Baer R, Ludwig T: **The BRCA1/BARD1 heterodimer, a tumor suppressor complex with ubiquitin E3 ligase activity.** *Curr Opin Genet Dev* 2002, **12**:86–91.
- Zhu Q, Pao GM, Huynh AM, Suh H, Tonnu N, Nederlof PM, Gage FH, Verma IM: **BRCA1 tumour suppression occurs via heterochromatin-mediated silencing.** *Nature* 2011, **477**:179–184.
- Linger RJ, Kruk PA: **BRCA1 16 years later: risk-associated BRCA1 mutations and their functional implications.** *FEBS J* 2010, **277**:3086–3096.
- Callebaut I, Mornon JP: **From BRCA1 to RAP1: a widespread BRCT module closely associated with DNA repair.** *FEBS Lett* 1997, **400**:25–30.
- Rodriguez M, Yu X, Chen J, Songyang Z: **Phosphopeptide binding specificities of BRCA1 COOH-terminal (BRCT) domains.** *J Biol Chem* 2003, **278**:52914–52918.
- Shakya R, Reid LJ, Reczek CR, Cole F, Egli D, Lin CS, deRooij DG, Hirsch S, Ravi K, Hicks JB, Szabolcs M, Jasin M, Baer R, Ludwig T: **BRCA1 tumor suppression depends on BRCT phosphoprotein binding, but not its E3 ligase activity.** *Science* 2011, **334**:525–528.
- Di Cecco L, Melissari E, Mariotti V, Iofrida C, Galli A, Guidugli L, Lombardi G, Caligo MA, Iacopetti P, Pellegrini S: **Characterisation of gene expression profiles of yeast cells expressing BRCA1 missense variants.** *Eur J Cancer* 2009, **45**:2187–2196.
- Guidugli L, Rugani C, Lombardi G, Aretini P, Galli A, Caligo MA: **A recombination-based method to characterize human BRCA1 missense variants.** *Breast Cancer Res Treat* 2011, **125**:265–272.
- Olopade OL, Fackenthal JD, Dunston G, Tainsky MA, Collins F, Whitfield-Broome C: **Breast cancer genetics in African Americans.** *Cancer* 2003, **97**(Suppl 1):236–245.
- 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**:123–133.
- Smyth G: **Linear models for microarray data.** In *Bioinformatics and computational biology solutions using R and Bioconductor*. Edited by Gentleman R, Carey V, Dudoit S, Izriary R, Huber W. New York: Springer; 2005:397–420.
- Lonnstedt I, Speed T: **Replicated microarray data.** *Stat Sinica* 2002, **12**:31–46.
- Draghici S, Khatri P, Tarca AL, Amin K, Done A, Voichita C, Georgescu C, Romero R: **A systems biology approach for pathway level analysis.** *Genome Res* 2007, **17**:1537–1545.
- Pathway-Express*. <http://vortex.cs.wayne.edu/projects.htm>.
- Khatri P, Draghici S, Ostermeier GC, Krawetz SA: **Profiling gene expression using onto-express.** *Genomics* 2002, **79**:266–270.
- Onto-Express*; [<http://vortex.cs.wayne.edu/projects.htm>].
- Coremine*; [<http://www.coremine.com/medical>].
- Bustin SA, Benes V, Garson JA, Hellemans J, Huggett J, Kubista M, Mueller R, Nolan T, Pfaffl MW, Shipley GL, Vandesompele J, Wittwer CT: **The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments.** *Clin Chem* 2009, **55**:611–622.
- Vandesompele J, De Preter K, Pattyn F, Poppe B, Van Roy N, De Paepe A, Speleman F: **Accurate normalization of real-time quantitative RT-PCR data by geometric averaging of multiple internal control genes.** *Genome Biol* 2002, **3**:research0034.1–research0034.11.
- ArrayExpress*; [<http://www.ebi.ac.uk/arrayexpress/>].
- Strobl J, Wonderlin W, Flynn D: **Mitogenic signal transduction in human breast cancer cells.** *Gen Pharmacol* 1995, **26**:1643–1649.
- Vermeulen K, Van Bockstaele DR, Berneman ZN: **The cell cycle: a review of regulation, deregulation and therapeutic targets in cancer.** *Cell Prolif* 2003, **36**:131–149.
- Zafonte BT, Hult J, Amanatullah DF, Albanese C, Wang C, Rosen E, Reutens A, Sparano JA, Lisanti MP, Pestell RG: **Cell-cycle dysregulation in breast cancer: breast cancer therapies targeting the cell cycle.** *Front Biosci* 2000, **5**:D938–961.
- Cazzalini O, Scovassi AI, Savio M, Stivala LA, Prosperi E: **Multiple roles of the cell cycle inhibitor p21(CDKN1A) in the DNA damage response.** *Mutat Res* 2010, **704**:12–20.
- Harris TE, Albrecht JH, Nakanishi M, Darlington GJ: **CCAAT/enhancer-binding protein-alpha cooperates with p21 to inhibit cyclin-dependent kinase-2 activity and induces growth arrest independent of DNA binding.** *J Biol Chem* 2001, **276**:29200–29209.
- Pardali K, Kowanzet M, Heldin CH, Moustakas A: **Smad pathway-specific transcriptional regulation of the cell cycle inhibitor p21(WAF1/Cip1).** *J Cell Physiol* 2005, **204**:260–272.
- Tian F, DaCosta Byfield S, Parks WT, Yoo S, Felici A, Tang B, Piek E, Wakefield LM, Roberts AB: **Reduction in Smad2/3 signaling enhances tumorigenesis but suppresses metastasis of breast cancer cell lines.** *Cancer Res* 2003, **63**:8284–8292.
- Kohn EA, Du Z, Sato M, Van Schyndle CM, Welsh MA, Yang YA, Stuelten CH, Tang B, Ju W, Bottinger EP, Wakefield LM: **A novel approach for the generation of genetically modified mammary epithelial cell cultures yields new insights into TGFβ signaling in the mammary gland.** *Breast Cancer Res* 2010, **12**:R83.
- Huang SM, Lu KT, Wang YC: **ATM/ATR and SMAD3 pathways contribute to 3-indole-induced G<sub>1</sub> arrest in cancer cells and xenograft models.** *Anticancer Res* 2011, **31**:203–208.
- Zelivianski S, Cooley A, Kall R, Jeruss JS: **Cyclin-dependent kinase 4-mediated phosphorylation inhibits Smad3 activity in cyclin D-overexpressing breast cancer cells.** *Mol Cancer Res* 2010, **8**:1375–1387.
- Li H, Sekine M, Seng S, Avraham S, Avraham HK: **BRCA1 interacts with Smad3 and regulates Smad3-mediated TGF-beta signaling during oxidative stress responses.** *PLoS One* 2009, **4**:e7091.
- Carbone R, Pearson M, Minucci S, Pelicci PG: **PML NBs associate with the hMre11 complex and p53 at sites of irradiation induced DNA damage.** *Oncogene* 2002, **21**:1633–1640.
- Ikura T, Ogryzko W, Grigoriev M, Groisman R, Wang J, Horikoshi M, Scully R, Qin J, Nakatani Y: **Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis.** *Cell* 2000, **102**:463–473.
- Han SH, Jeon JH, Ju HR, Jung U, Kim KY, Yoo HS, Lee YH, Song KS, Hwang HM, Na YS, Yang Y, Lee KN, Choi I: **VDUP1 upregulated by TGF-beta1 and 1,25-dihydroxyvitamin D3 inhibits tumor cell growth by blocking cell-cycle progression.** *Oncogene* 2003, **22**:4035–4046.
- Burbee D, Forgacs E, Zöchbauer-Müller S, Shivakumar L, Fong K, Gao B, Randle D, Kondo M, Virmani A, Bader S, Sekido Y, Latif F, Milchgrub S, Toyooka S, Gazdar AF, Lerman MI, Zbarovsky E, White M, Minna JD: **Epigenetic inactivation of RASSF1A in lung and breast cancers and malignant phenotype suppression.** *J Natl Cancer Inst* 2001, **93**:691–699.
- Shivakumar L, Minna J, Sakamaki T, Pestell R, White M: **The RASSF1A tumor suppressor blocks cell cycle progression and inhibits cyclin D1 accumulation.** *Mol Cell Biol* 2002, **22**:4309–4318.
- Shaulian E, Karin M: **AP-1 in cell proliferation and survival.** *Oncogene* 2001, **20**:2390–2400.
- Nunes-Xavier C, Romá-Mateo C, Ríos P, Tárrega C, Cejudo-Marín R, Taberner L, Pulido R: **Dual-Specificity MAP Kinase Phosphatases as**

- Targets of Cancer Treatment.** *Anticancer Agents Med Chem* 2011, **11**:109–132.
43. Small GW, Shi YY, Edmund NA, Somasundaram S, Moore DT, Orlowski RZ: **Evidence that mitogen-activated protein kinase phosphatase-1 induction by proteasome inhibitors plays an antiapoptotic role.** *Mol Pharmacol* 2004, **66**:1478–1490.
44. Wang HY, Cheng Z, Malbon CC: **Overexpression of mitogen-activated protein kinase phosphatases MKP1, MKP2 in human breast cancer.** *Cancer Lett* 2003, **191**:229–237.
45. Givant-Horwitz V, Davidson B, Goderstad JM, Nesland JM, Tropé CG, Reich R: **The PAC-1 dual specificity phosphatase predicts poor outcome in serous ovarian carcinoma.** *Gynecol Oncol* 2004, **93**:517–523.
46. Bagnato A, Rosanò L: **The endothelin axis in cancer.** *Int J Biochem Cell Biol* 2008, **40**:1443–1451.
47. Bassermann F, Pagano M: **Dissecting the role of ubiquitylation in the DNA damage response checkpoint in G2.** *Cell Death Differ* 2010, **17**:78–85.
48. Kops GJ, Kim Y, Weaver BA, Mao Y, McLeod I, Yates JR, Tagaya M, Cleveland DW: **ZW10 links mitotic checkpoint signaling to the structural kinetochore.** *J Cell Biol* 2005, **169**:49–60.
49. Xu L, Begum S, Hearn JD, Hynes RO: **GPR56, an atypical G protein-coupled receptor, binds tissue transglutaminase, TG2, and inhibits melanoma tumor growth and metastasis.** *Proc Natl Acad Sci U S A* 2006, **103**:9023–9028.
50. Shishodia S, Aggarwal BB: **Nuclear factor-kappaB: a friend or a foe in cancer?** *Biochem Pharmacol* 2004, **68**:1071–1080.
51. Rayet B, Gélinas C: **Aberrant rel/nfkb genes and activity in human cancer.** *Oncogene* 1999, **18**(49):6938–6947.
52. Kühnel F, Zender L, Paul Y, Tietze MK, Trautwein C, Manns M, Kubicka S: **NFkappaB mediates apoptosis through transcriptional activation of Fas (CD95) in adenoviral hepatitis.** *J Biol Chem* 2000, **275**:6421–6427.
53. Shetty S, Graham BA, Brown JG, Hu X, Vegh-Yarema N, Harding G, Paul JT, Gibson SB: **Transcription factor NF-kappaB differentially regulates death receptor 5 expression involving histone deacetylase 1.** *Mol Cell Biol* 2005, **25**:5404–5416.
54. Sheridan JP, Marsters SA, Pitti RM, Gurney A, Skubatch M, Baldwin D, Ramakrishnan L, Gray CL, Baker K, Wood WI, Goddard AD, Godowski P, Ashkenazi A: **Control of TRAIL-induced apoptosis by a family of signaling and decoy receptors.** *Science* 1997, **277**:818–821.
55. Suliman A, Lam A, Datta R, Srivastava RK: **Intracellular mechanisms of TRAIL: apoptosis through mitochondrial-dependent and -independent pathways.** *Oncogene* 2001, **20**:2122–2133.
56. Taira N, Nihira K, Yamaguchi T, Miki Y, Yoshida K: **DYRK2 is targeted to the nucleus and controls p53 via Ser46 phosphorylation in the apoptotic response to DNA damage.** *Mol Cell* 2007, **25**:725–738.
57. Chen W, Li N, Chen T, Han Y, Li C, Wang Y, He W, Zhang L, Wan T, Cao X: **The lysosome-associated apoptosis-inducing protein containing the pleckstrin homology (PH) and FYVE domains (LAPF), representative of a novel family of PH and FYVE domain-containing proteins, induces caspase-independent apoptosis via the lysosomal-mitochondrial pathway.** *J Biol Chem* 2005, **280**:40985–40995.
58. Li N, Zheng Y, Chen W, Wang C, Liu X, He W, Xu H, Cao X: **Adaptor protein LAPF recruits phosphorylated p53 to lysosomes and triggers lysosomal destabilization in apoptosis.** *Cancer Res* 2007, **67**:11176–11185.
59. Khanna KK, Jackson SP: **DNA double-strand breaks: signaling, repair and the cancer connection.** *Nat Genet* 2001, **27**:247–254.
60. Martin RW, Orelli BJ, Yamazoe M, Minn AJ, Takeda S, Bishop DK: **RAD51 up-regulation bypasses BRCA1 function and is a common feature of BRCA1-deficient breast tumors.** *Cancer Res* 2007, **67**:9658–9665.
61. Saviozzi S, Ceppi P, Novello S, Ghio P, Lo Iacono M, Borasio P, Cambieri A, Volante M, Papotti M, Calogero RA, Scagliotti GV: **Non-small cell lung cancer exhibits transcript overexpression of genes associated with homologous recombination and DNA replication pathways.** *Cancer Res* 2009, **69**:3390–3396.
62. Dever SM, Golding SE, Rosenberg E, Adams BR, Idowu MO, Quillin JM, Valerie N, Xu B, Povirk LF, Valerie K: **Mutations in the BRCT binding site of BRCA1 result in hyper-recombination.** *Ageing (Albany NY)* 2011, **3**:515–532.
63. Park BJ, Kang JW, Lee SW, Choi SJ, Shin YK, Ahn YH, Choi YH, Choi D, Lee KS, Kim S: **The haploinsufficient tumor suppressor p18 upregulates p53 via interactions with ATM/ATR.** *Cell* 2005, **120**:209–221.
64. Sumara I, Vorlaufer E, Gieffers C, Peters BH, Peters JM: **Characterization of vertebrate cohesin complexes and their regulation in prophase.** *J Cell Biol* 2000, **151**:749–762.
65. Yazdi P, Wang Y, Zhao S, Patel N, Lee E, Qin J: **SMC1 is a downstream effector in the ATM/NBS1 branch of the human S-phase checkpoint.** *Genes Dev* 2002, **16**:571–582.
66. Connor J, Weiser D, Li S, Hallenbeck J, Shenolikar S: **Growth arrest and DNA damage-inducible protein GADD34 assembles a novel signaling complex containing protein phosphatase 1 and inhibitor 1.** *Mol Cell Biol* 2001, **21**:6841–6850.
67. Shtivelman E, Cohen FE, Bishop JM: **A human gene (AHNAK) encoding an unusually large protein with a 1.2-microns polyionic rod structure.** *Proc Natl Acad Sci U S A* 1992, **89**:5472–5476.
68. Stiff T, Shtivelman E, Jeggo P, Kysela B: **AHNAK interacts with the DNA ligase IV-XRCC4 complex and stimulates DNA ligase IV-mediated double-stranded ligation.** *DNA Repair (Amst)* 2004, **3**:245–256.
69. Oberley TD, Oberley LW: **Antioxidant enzyme levels in cancer.** *Histol Histopathol* 1997, **12**:525–535.
70. Bravard A, Hoffschir F, Sabatier L, Ricou M, Pinton A, Cassingena R, Estrade S, Luccioni C, Dutrillaux B: **Early superoxide dismutase alterations during SV40-transformation of human fibroblasts.** *Int J Cancer* 1992, **52**:797–801.
71. Li JJ, Oberley LW, St Clair DK, Ridnour LA, Oberley TD: **Phenotypic changes induced in human breast cancer cells by overexpression of manganese-containing superoxide dismutase.** *Oncogene* 1995, **10**:1989–2000.
72. 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.** *Genes Dev* 2000, **14**:927–939.
73. Zhang J, Powell S: **The role of the BRCA1 tumor suppressor in DNA double-strand break repair.** *Mol Cancer Res* 2005, **3**:531–539.
74. Kuimov AN: **Polypeptide components of telomere nucleoprotein complex.** *Biochemistry (Mosc)* 2004, **69**:117–129.
75. Richard DJ, Bolderson E, Cubeddu L, Wadsworth RL, Savage K, Sharma GG, Nicolette ML, Tsvetanov S, McLlwraith MJ, Pandita RK, White MF, Khanna KK: **Single-stranded DNA-binding protein hSSB1 is critical for genomic stability.** *Nature* 2008, **453**:677–681.

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