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GENETIC INSTABILITY IN THE *RAD51* AND *BRCA1* REGIONS IN BREAST CANCER

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Abstract: Breast cancer is the most prevalent cancer type in women. Accumulating evidence indicates that the fidelity of double-strand break repair in response to DNA damage is an important step in mammary neoplasias. The RAD51 and BRCA1 proteins are involved in the repair of double-strand DNA breaks by homologous recombination. In this study, we evaluated loss of heterozygosity (LOH) in the *RAD51* and *BRCA1* regions, and their association with breast cancer. The polymorphic markers D15S118, D15S214 and D15S1006 were the focus for *RAD51*, and D17S855 and D17S1323 for *BRCA1*. Genomic deletion detected by allelic loss varied according to the regions tested, and ranged from 29 to 46% of informative cases for the *RAD51* region and from 38 to 42% of informative cases for the *BRCA1* region. 25% of breast cancer cases displayed LOH for at least one studied marker in the *RAD51* region exclusively. On the other hand, 31% of breast cancer cases manifested LOH for at least one microsatellite marker concomitantly in the *RAD51* and *BRCA1* regions. LOH in the *RAD51* region,

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Abbreviations used: AML – acute myeloid leukemia; BACH1 helicase – BRCA1 interacting protein C-terminal helicase 1 [*Homo sapiens*]; *BRCA1* – breast cancer susceptibility gene 1; BRCT – BRCA C-terminus; LOH – loss of heterozygosity; RecA – RecA protein (Recombinase A); MRE11 – meiotic recombination 11 homologue A; NBS1 – Nijmegen breakage syndrome 1 (nibrin, NBN); p53 – tumor protein 53; *RAD50* – RAD50 homologue (*Saccharomyces cerevisiae*) [*Homo sapiens*]; *RAD51* – RAD51 homologue (RecA homologue, *Escherichia coli*) (*Saccharomyces cerevisiae*) [*Homo sapiens*]; *RAD52* – RAD52 homologue (*Saccharomyces cerevisiae*) [*Homo sapiens*]; t-AML – therapy related AML

similarly as in the *BRCA1* region, appeared to correlate with steroid receptor status. The obtained results indicate that alteration in the *RAD51* region may contribute to the disturbances of DNA repair involving *RAD51* and *BRCA1* and thus enhance the risk of breast cancer development.

Key words: *RAD51*, *BRCA1*, Loss of heterozygosity (LOH), Breast cancer

INTRODUCTION

Breast cancer occurs in both hereditary and sporadic forms, and is a great problem in public health all over the world. Although mutations in the *BRCA1* gene seem to be the most essential for familial and sporadic breast cancer, it has become clear that breast cancer is a complex phenomenon in which multiple genes may play a role. A wide variety of cellular pathway alterations may confer and increase the risk of breast cancer. Among them, the DNA damage response is of great importance. DNA repair is critical for maintaining genome integrity. The *BRCA1* gene product was found to be involved in the repair of double-strand DNA breaks by homologous recombination, particularly through the mechanism involving *RAD51*. Thus, *RAD51* may contribute to breast cancer by maintaining genomic integrity and/or modifying the penetrance of *BRCA1* mutations [1-4].

The *RAD51* gene located on chromosome 15q15.1 consists of 10 exons and 9 introns, and spans at least 30 kb [5]. The human *RAD51* gene encodes a 339-amino acid protein with a molecular weight of 37 kDa, a homologue of the RecA protein of *Escherichia coli* and Rad51 of *Saccharomyces cerevisiae*, and is involved in both meiotic and mitotic recombination. The RAD51 protein seems to be essential for maintaining genomic stability, and it plays a central role in the homology-dependent recombinational repair of DNA double-strand breaks [6, 7]. RAD51 binds to single and double-stranded DNA, exhibits DNA-dependent ATPase activity to form nucleoprotein filaments, and mediates homologous pairing and strand exchange between DNA duplexes [8-10]. RAD51 is expressed in proliferating cells with the highest level in the S or S/G_2 phase of the cell cycle [11-13]. Specific interaction between RAD51 and such proteins as BRCA1, BRCA2, p53 and RAD52 has been described [14-18]. BRCA1 was shown to bind with RAD51 and co-localize with RAD51 in mitotic and meiotic cells [19, 20].

BRCA1 is a tumor suppressor gene located on chromosome 17q21, and it spans 100 kb of genomic DNA [21]. The *BRCA1* gene encodes a nuclear phosphoprotein of 220 kDa consisting of 1863 amino acids, which has a highly conserved amino terminal RING finger domain and a C-terminal domain (BRCT) characteristic for many transcription factors [22-24]. Two BRCT motifs have been identified in BRCA1 and in several other proteins involved in cell-cycle control regulation in response to DNA damage [25, 26]. BRCA1 was found to be involved in several important cellular functions, including DNA

damage repair, transcription regulation, cell-cycle control, protein ubiqutination, apoptosis, and chromatin remodeling [27-30]. Some of these diverse functions are associated with a specific partner protein. BRCA1 interacts with multiple DNA repair/recombination proteins, including RAD51, the RAD50/MRE11/NBS1 complex, Bloom's helicase, BACH1 helicase and Fanconi's proteins [20, 31-36].

The accumulated data suggests that genetic instability of *BRCA1* is associated with an increased relative risk of breast cancer [37, 38]. The aim of this study was to evaluate if *RAD51* chromosomal region alteration contributes to breast cancer. We evaluated loss of heterozygosity (LOH) in the *RAD51* and *BRCA1* regions, and their association with breast cancer. The polymorphic markers D15S118, D15S214 and D15S1006 were the focus for *RAD51*, and D17S855 and D17S1323 for *BRCA1*. The relationship of LOH with clinicopathological parameters was examined to reveal the potential role of the studied genes in breast cancer development.

MATERIALS AND METHODS

Patients

Thirty six paraffin-embedded tissue samples from patients with primary breast cancer and matched blood samples were obtained at the Department of Clinical Pathomorphology of the Polish Mother's Memorial Hospital Research Institute, Łódź, Poland. The mean age of the patients was 57, ranging from 32 to 79. Fourteen were 50 years old or younger, and 22 were over the age of 50. All the tumor specimens underwent clinicohistopathological evaluations. All were classified as ductal carcinoma. The series included 35 cases at stage II and 1 at stage III, according to the modified Bloom-Richardson criteria. Twenty seven cases were positive and 9 negative with respect to estrogen receptors, 17 were positive and 19 negative with respect to progesterone receptors, and 20 cases were negative and 16 positive with respect to lymph node status.

DNA isolation

DNA was isolated from peripheral blood and tissue samples following the standard phenol extraction procedure. The paraffin-embedded tissue samples were extracted with xylene to remove the paraffin [39, 40].

PCR conditions and primers

The specimens were investigated for genetic alterations at the two genetic regions using 5 microsatellite markers. For *RAD51*, we focused on the polymorphic microsatellite markers D15S118, D15S214 and D15S1006, oriented along the chromosomal region 15q14-q21. The localization of the studied markers is as follows: D15S118–12996900-12997100 bp, D15S214–17166170-17166435 bp, D15S1006–24439646-24439859 bp. The *RAD51* gene is located between 17791587 and 17792320 bp (NCBI, Gene Map, Celera). The marker D15S118 is at the centromeric position, D15S214 at the middle, and D15S1006 at the

telomeric, relative to the *RAD51* gene. For *BRCA1*, we focused on the intragenic microsatellite markers D17S855 and D17S1323 (intron 20 and 12) (J. Weissenbach, Genethon, Whitehead Institute Center for Genome Research). The sequences of primers used for PCR are shown in Tab. 1. The sequences for all the primers are listed in the Human Genome Database (www.gdb.org). The primers were synthesized and labeled fluorescently by Applied Biosystems (USA). Polymerase chain reaction (PCR) was carried out in a 7.5 µl reaction volume containing 50 ng of genomic DNA, 0.3 units of AmpliTaq Gold™ DNA polymerase (5 U/µl), 1 x GeneAmp® PCR Gold Buffer (10 x concentration), 1 mM GeneAmp dNTP Mix (10 mM), 2.5 mM magnesium chloride (25 mM) and 5 pmol of either forward or reverse primer end-labeled with the dye phosphoramidite 6-FAM or TET. A 30-cycle amplification (denaturation, annealing and extension) was done in a GeneAmp 2400 thermal cycler (Perkin-Elmer, USA). The PCR cycles for each marker are presented in Tab. 1.

Tab. 1. Characteristics of the microsatellite markers analyzed.

Microsatellite marker	Primer sequences (5'→3')	PCR conditions
D15S118	TCA AAG ACC CAT ATC AACC GTG CTG AAA AGC GAC ACTT	
D15S214	GGA GGG CAC TTC CTG AG GCC TGG CAT CAC GACT	30 cycles of 94ºC for 15 s, 55ºC for 30 s, 72ºC for 30 s
D15S1006	AGG GAA TAC TTC AAA ACTC CCA CTT GGC TAT GGT GAAT	
D17S855	ACA CAG ACT TGT CCT ACT GCC GGA TGG CCT TTT AGA AAG TGG	30 cycles of 94ºC for 15 s, 51ºC for 30 s, 72ºC for 30 s
D17S1323	TAG GAG ATG GAT TAT TGG TG AAG CAA CTT TGC AAT GAG TG	30 cycles of 94ºC for 15 s, 56ºC for 30 s, 72ºC for 30 s

LOH analysis

PCR products were analyzed on 5% polyacrylamide gel (5% Long Ranger) containing 6 M urea and 1 x TBE (10 x TBE: 0.89 M Tris borate, 0.02 M EDTA, pH 8.0). Samples of 3 µl of reaction mixture were mixed with 4 µl of stop solution containing ten parts deionized formamide, two parts GeneScan™-350 TAMRA Size Standard and one part loading buffer (50 mg/ml blue dextran, 25 mM EDTA). Samples were denatured at 95ºC for 5 min and chilled on ice. 3 µl of each sample was loaded in the well of the gel and run for 2 h in an ABI PRISM 377™ DNA Sequencer (Applied Biosystems, USA). The data was collected

automatically. Allele sizing was determined with GeneScan version 3.1.2 and Genotyper version 2.5 softwares (Applied Biosystems, USA), and also calculated as described by Cawkwell *et al.* [41], with reciprocal correction as required. LOH was defined as ≥ 50% reduction (allelic ratio ≤ 0.5) in either allele in the tumor compared with the normal counterpart.

Statistical analysis

All the comparisons between LOH and clinicopathological parameters were performed using the Fisher test. *P*-values of 0.05 or less were considered statistically significant. The statistical analysis was performed using the Statistica package, version 5.

RESULTS

The LOH analysis of the *RAD51* and *BRCA1* regions was performed on the microsatellite markers D15S118, D15S214 and D15S1006 for the former, and D17S855 and D17S1323 for the latter, using DNA isolated from the tumor and matched peripheral blood of 36 breast cancer patients. The LOH study detected the loss of a single copy of the two alleles. Those with detectable heterozygous alleles are defined as informative cases. As shown in Tab. 2, genomic deletion detected by allelic loss varied according to the region tested, and ranged from 29% (6/21) to 46% (12/26) of informative cases for the *RAD51* region and from 38% (8/21) to 42% (11/26) of informative cases for the *BRCA1* region. A high incidence of LOH (41%) was observed for the highly informative microsatellite marker D15S214, which is located near the *RAD51* locus. 25% (9/36) of the studied breast cancer cases displayed LOH for at least one microsatellite marker in the *RAD51* region. 31% (11/36) of breast cancer cases manifested LOH for at least one microsatellite marker concomitantly in the *RAD51* and *BRCA1* regions.

Tab. 2. The incidence of LOH in the *RAD51* and *BRCA1* regions in breast cancer.

Gene/Chromosomal localization	Microsatellite marker	Informative cases (%)	Tumors with LOH (%)
RAD51 15q15.1	D15S118	26/36 (72)	12/26 (46)
	D15S214	17/36 (47)	7/17 (41)
	D15S1006	21/36 (58)	6/21 (29)
BRCA1 17q21	D17S855	21/36 (58)	8/21 (38)
	D17S1323	26/36 (72)	11/26 (42)

The LOH in the *RAD51* and *BRCA1* regions and clinicopathological parameters were compared using the Fisher test. The LOH at D15S118 and D15S214 was found to be significantly more frequent in the estrogen-receptor positive than in the estrogen-receptor negative patients ($P = 0.03$ and $P = 0.02$). There was a trend towards statistical significance in the frequency of LOH at D15S118,

Tab. 3. The relationship between LOH in the *RAD51* chromosomal region and clinico-pathological parameters in breast cancer.

Characteristics	Microsatellite marker												
	T	D15S118				D15S214				D15S1006			
		I	N	LOH	*P*	I	N	LOH	*P*	I	N	LOH	*P*
Tumor cases	36	26	14	12	—	17	10	7	—	21	15	6	—
Patients' age					NS				NS				NS
≤ 50	14	11	4	7		5	2	3		8	4	4	
> 50	22	15	10	5		12	8	4		13	11	2	
Tumor grade					—				—				NS
II	35	26	14	12		17	10	7		20	15	5	
III	1	—	—	—		—	—	—		1	—	1	
Estrogen receptor					0.03				0.02				NS
Positive	27	21	14	7		8	2	6		14	11	3	
Negative	9	5	—	5		9	8	1		7	4	3	
Progesterone receptor					0.01				0.02				0.01
Positive	17	10	2	8		8	2	6		8	3	5	
Negative	19	16	12	4		9	8	1		13	12	1	
Nodal status					NS				NS				NS
Negative	20	17	8	9		11	7	4		15	10	5	
Positive	16	9	6	3		6	3	3		6	5	1	

T – Total number of tumors studied; I – Number of informative cases; N – Heterozygous without LOH; *P* – Fisher test.

Tab. 4. The relationship between LOH in the *BRCA1* chromosomal region and clinico-pathological parameters in breast cancer.

Characteristics	Microsatellite marker								
	T	D17S855				D17S1323			
		I	N	LOH	*P*	I	N	LOH	*P*
Tumor cases	36	21	13	8	—	26	15	11	—
Patients' age					0.01				0.01
≤ 50	14	6	1	5		9	2	7	
> 50	22	15	12	3		17	13	4	
Tumor grade					—				—
II	35	21	13	8		26	15	11	
III	1	—	—	—		—	—	—	
Estrogen receptor					0.04				NS
Positive	27	15	7	8		17	8	9	
Negative	9	6	6	—		9	7	2	
Progesterone receptor					0.03				0.04
Positive	17	9	3	6		10	3	7	
Negative	19	12	10	2		16	12	4	
Nodal status					0.04				0.04
Negative	20	18	13	5		12	4	8	
Positive	16	3	—	3		14	11	3	

T – Total number of tumors studied; I – Number of informative cases; N – Heterozygous without LOH; *P* – Fisher test.

D15S214 and D15S1006 with positive progesterone receptor status (P = 0.01, P = 0.02 and P = 0.01, respectively) (Tab. 3). LOH at D17S855 and D17S1323 also occurred frequently in tumors with positive progesterone receptor (P = 0.03, P = 0.04) and negative nodal status (P = 0.04) compared to those with negative progesterone receptor and positive nodal status. Furthermore, the incidence of LOH at D17S855 was also associated with positive estrogen receptor status (P = 0.04) (Tab. 4). These results seem to suggest that genetic instability in the *RAD51* and *BRCA1* regions occurs early in mammary carcinogenesis.

DISCUSSION

Genomic instability is one of the main features of cancer cells. It is expressed by the accumulation of chromosomal aberrations, mutations, loss of heterozygosity and microsatellite instability. LOH is observed in the early and late stages of the neoplastic transformation process [42]. Because of the high level of specificity, LOH has recently become invaluable as a marker for the diagnosis and prognosis of cancer [43].

The repair of chromosomal double-strand breaks is essential to maintain genomic integrity, yet the various repair pathways are variably mutagenic. RAD51 and BRCA1 proteins are involved in double-strand break repair by homologous recombination [44, 45]. Single nucleotide polymorphisms have been identified in the 5' untranslated region of *RAD51*, namely 5'UTRg135c and 5'UTRg172t [46]. *RAD51*-135c itself has not been demonstrated to elevate the risk of breast cancer [47-50]. It is not clear whether *BRCA1* mutation carriers, which also carried the RAD51-135c variant, had a higher susceptibility of developing breast cancer compared with *BRCA1* mutation carriers without this single nucleotide polymorphism. Wang *et al.* [46] suggested that single nucleotide polymorphisms in the *RAD51* 5' untranslated region might be associated with an increased risk of breast cancer among *BRCA1* mutation carriers. A matched case study of Polish women showed instead that *RAD51*-135c is associated with a decreased risk of breast cancer in women who carry the *BRCA1* mutation 5382insC [51]. No or a low association was detected between epithelial ovarian cancer risk and *RAD51* g135c and *RAD51* g172t [3, 46]. On the other hand, both *de novo* and therapy-related acute myeloid leukemia (AML and t-AML) have been found to be associated with *RAD51*-135c polymorphism [52]. Schmutte *et al.* [5] did not find any mutations in the *RAD51* coding sequence or intron/exon boundaries, or hypermethylation in the promoter region in breast cancer and metastatic brain tumors. A sequence analysis of the coding region of the *RAD51* cDNA demonstrated no point mutations or microdeletions in the parathyroid cancer [53]. Kato *et al.* [54] found a missense mutation in two patients with familial breast cancer: a G-to-A transition converting codon 150 from CGG (Arg) to CAG (Gln). Both patients had bilateral breast cancer, one with synchronous bilateral breast cancer and the other with synchronous bilateral multiple breast cancer.

Loss of heterozygosity in the genomic region 15q14-q21, containing *RAD51*, has been reported in 32-70% of breast cancer cases [55-57], 56% of lung cancer [55], 67% of colorectal cancer [55], 46-54% of malignant mesothelioma [58, 59], and 39% of bladder transitional cell carcinoma [60]. LOH at 17q21 has been revealed in about 30-60% of breast [61, 62], ovarian [63] and colorectal [64] cancer cases. Gonzalez *et al.* [56] observed LOH at the *RAD51* and *BRCA1* regions for at least one marker, respectively in 32% and 49% of breast cancers. In our study, 25% of breast cancer cases displayed LOH for at least one microsatellite marker in the *RAD51* region exclusively. On the other hand, 31% of cases manifested LOH for at least one microsatellite marker both in the *RAD51* and *BRCA1* regions. The obtained results suggest that *RAD51* alterations may play a critical role in genomic instability due to the lack of efficiency of DNA repair involving the *RAD51* and *BRCA1* genes.

Recent studies indicated that allelic loss in the aforementioned regions might be associated with clinicopathological features of breast cancer. Statistically significant differences between breast tumors with and without LOH in the *RAD51* and *BRCA1* regions have been found with respect to estrogen receptor content, progesterone receptor content, higher grade, and stage [56, 65, 66]. Johnson *et al.* [62] detected allelic loss of BRCA1 with higher frequency in women under 36 compared to postmenopausal patients. However, this difference was not statistically significant. On the contrary, Santos *et al.* [67] observed no correlation when LOH frequency in the BRCA1 region was compared with tumor size or grade, or the presence of axillary lymph node metastasis. In our study, when LOH in the *RAD51* and *BRCA1* regions was correlated with the clinicopathological parameters of breast cancer, we noticed statistically significant differences mainly between tumors with LOH and estrogen and progesterone receptor status. These results indicate that further studies are needed to establish more specific association of LOH in the *RAD51* and *BRCA1* regions with clinicopathological parameters in breast cancer.

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REFERENCES

1. Nathanson, K.L. and Weber, B.L. "Other" breast cancer susceptibility genes: searching for more holy grail. **Hum. Mol. Genet.** 10 (2001) 715-720.
2. Welcsh, P.L. and King, M.C. BRCA1 and BRCA2 and the genetics of breast and ovarian cancer. **Hum. Mol. Genet.** 10 (2001) 705-713.
3. Auranen, A., Song, H., Waterfall, C., Dicioccio, R.A., Kuschel, B., Kjaer, S.K., Hogdall, E., Hogdall, C., Stratton, J., Whittemore, A.S., Easton, D.F., Ponder, B.A., Novik, K.L., Dunning, A.M., Gayther, S. and Pharoah, P.D. Polymorphisms in DNA repair genes and epithelial ovarian cancer risk. **Int. J. Cancer** 117 (2005) 611-618.

4. Cousineau, I., Abaji C. and Belmaaza, A. BRCA1 regulates RAD51 function in response to DNA damage and suppresses spontaneous sister chromatid replication slippage: implications for sister chromatid cohesion, genome stability, and carcinogenesis. **Cancer Res.** 65 (2005) 11384-11391.
5. Schmutte, C., Tombline, G., Rhiem, K., Sadoff, M.M., Schmutzler, R., von Deimling, A. and Fishel, R. Characterization of the human *Rad51* genomic locus and examination of tumors with 15q14-15 loss of heterozygosity (LOH). **Cancer Res.** 59 (1999) 4564-4569.
6. Shinohara, A., Ogawa, H., Matsuda, Y., Ushio, N., Ikoe, K. and Ogawa, T. Cloning of human, mouse and fission yeast recombination genes homologous to *RAD51* and *recA*. **Nature Genet**. 4 (1993) 239-243.
7. Takahashi, E., Matsuda, Y., Hori, T., Yasuda, N., Tsuji, S., Mori, M., Yoshimura, Y., Yamamoto, A., Morita, T. and Matsushiro, A. Chromosome mapping of the human (*RECA*) and mouse (*Reca*) homologs of the yeast *RAD51* and *Escherichia coli recA* genes to human (15q15.1) and mouse (2F1) chromosomes by direct R-banding fluorescence *in situ* hybridization. **Genomics** 19 (1994) 376-378.
8. Benson, F.E., Stasiak, A. and West S.C. Purification and characterization of the human Rad51 protein, an analogue of *E. coli* RecA. **EMBO J.** 13 (1994) 5764-5771.
9. Baumann, P., Benson, F.E. and West, S.C. Human Rad51 protein promotes ATP-dependent homologous pairing and strand transfer reactions in vitro. **Cell** 87 (1996) 757-766.
10. Gupta, R., Bazemore, L.R., Golub, E.I. and Radding, C.M. Activities of human recombination protein Rad51. Proc **Natl. Acad. Sci. USA** 94 (1997) 463-468.
11. Yamamoto, A., Taki, T., Yagi, H., Habu, T., Yoshida, K., Yoshimura, Y., Yamamoto, K., Matsushiro, A., Nishimune, Y. and Morita, T. Cell cycle-dependent expression of the mouse Rad51 gene in proliferating cells. **Mol. Gen. Genet.** 251 (1996) 1-12.
12. Chen, F., Nastasi, A., Shen, Z., Brenneman, M., Crissman, H. and Chen, D.J. Cell cycle-dependent protein expression of mammalian homologs of yeast DNA double-strand break repair genes Rad51 and Rad52. **Mutat. Res.** 384 (1997) 205-211.
13. Xia, S.J., Shammas, M.A. and Shmookler Reis, R.J. Elevated recombination in immortal human cells is mediated by HsRAD51 recombinase. **Mol. Cell. Biol.** 17 (1997) 7151-7158.
14. Shen, Z., Cloud, K.G., Chen, D.J. and Park, M.S. Specific interactions between the human RAD51 and RAD52 proteins. **J. Biol. Chem.** 271 (1996) 148-152.
15. Sturzbecher, H.W., Donzelmann, B., Henning, W., Knippschild, U. and Buchhop, S. p53 is linked directly to homologous recombination processes via RAD51/RecA protein interaction. **EMBO J.** 15 (1996) 1992-2002.

16. Buchhop, S., Gibson, M.K., Wang, X.W., Wagner, P., Sturzbecher, H.W. and Harris, C.C. Interaction of p53 with the human Rad51 protein. **Nucleic Acids Res.** 25 (1997) 3868-3874.
17. Wong, A.K., Pero, R., Ormonde, P.A., Tavtigian, S.V. and Bartel, P.L. RAD51 interacts with the evolutionarily conserved BRC motifs in the human breast cancer susceptibility gene brca2. **J. Biol. Chem.** 272 (1997) 31941-31944.
18. Marmorstein, L.Y., Ouchi, T. and Aaronson, S.A. The BRCA2 gene product functionally interacts with p53 and RAD51. **Proc. Natl. Acad. Sci. USA.** 95 (1998) 13869-13874.
19. Scully, R. Chen, J., Plug, A., Xiao, Y., Weaver, D., Feunteun, J., Ashley, T. and Livingston, D.M. Association of BRCA1 with Rad51 in mitotic and meiotic cells. **Cell** 188 (1997) 265-275.
20. Zhong, Q., Chen, C.F., Li, S., Chen, Y., Wang, C.C., Xiao, J., Chen, P.L., Sharp, Z.D. and Lee, W.H. Association of BRCA1 with the hRad50--hMre11-p95 complex and the DNA damage response. **Science** 285 (1999) 747-750.
21. Hall, J.M., Lee, M.K., Newman, B., Morrow, J.E., Anderson, L.A., Huey, B. and King, M.C. Linkage of early-onset familial breast cancer to chromosome 17q21. **Science** 250 (1990) 1684-1689.
22. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., Liu, Q., Cochran, C., Bennett, L.M., Ding, W. Bell, R., Rosenthal, J., Hussey, C., Tran, T., McClure, M., Frye, C., Hattier, T., Phelps, R., Haugen-Strano, A., Katcher, H., Yakumo, K., Gholami, Z., Shaffer, D., Stone, S., Bayer, S., Wray, C., Bogden, R., Dayananth, P., Ward, J., Tonin, P., Narod, S., Bristow, P.K., Norris, F.H. Helvering, L., Morrison, P., Rosteck, P., Lai, M., Barrett, J.C., Lewis, C., Neuhausen, S., Cannon-Albright, L., Goldgar, D., Wiseman, R., Kamb, A., Skolnick, M.H. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. **Science** 266 (1994) 66-71.
23. Monteiro, A.N., August, A. and Hanafusa, H. Evidence for a trans-criptional activation function of BRCA1 C-terminal region. **Proc. Natl. Acad. Sci. USA** 93 (1996) 13595-13599.
24. Huyton, T., Bates, P.A., Zhang, X., Sternberg, M.J. and Freemont, P.S. The BRCA1 C-terminal domain: structure and function. **Mutat. Res.** 460 (2000) 319-332.
25. Koonin, E.V., Altschul, S.F. and Bork, P. BRCA1 protein products … Functional motifs… . **Nat. Genet.** 13 (1996) 266-268.
26. Bork, P., Hofmann, K., Bucher, P., Neuwald, A.F., Altschul, S.F.and Koonin, E.V. A superfamily of conserved domains in DNA damage-responsive cell cycle checkpoint proteins. **FASEB J.** 11 (1997) 68-76.
27. Larson, J.S., Tonkinson, J.L. and Lai, M.T. A BRCA1 mutant alters G2-M cell cycle control in human mammary epithelial cells. **Cancer Res.** 57 (1997) 3351-3355.

28. Moynahan, M.E., Chiu, J.W., Koller, B.H. and Jasin, M. Brca1 controls homology-directed DNA repair. **Mol. Cell** 4 (1999) 511-518.
29. Scully, R. and Livingston, D.M. In search of the tumour-suppressor functions of BRCA1 and BRCA2. **Nature** 408 (2000) 429-432.
30. Wang, Y., Cortez, D., Yazdi, P., Neff, N., Elledge, S.J. and Qin, J. BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. **Genes Dev.** 14 (2000) 927-939.
31. Cantor, S.B., Bell, D.W., Ganesan, S., Kass, E.M., Drapkin, R., Grossman, S., Wahrer, D.C., Sgroi, D.C., Lane, W.S., Haber, D.A. and Livingston, D.M. BACH1, a novel helicase-like protein, interacts directly with BRCA1 and contributes to its DNA repair function. **Cell** 105 (2001) 149-160.
32. Garcia-Higuera, I., Taniguchi, T., Ganesan, S., Meyn, M.S., Timmers, C., Hejna, J., Grompe, M. and D'Andrea, A.D. Interaction of the Fanconi anemia proteins and BRCA1 in a common pathway. **Mol. Cell** 7 (2001) 249-262.
33. Franchitto, A. and Pichierri, P. Bloom's syndrome protein is required for correct relocalization of RAD50/MRE11/NBS1 complex after replication fork arrest. **J. Cell Biol.** 157 (2002) 19-30.
34. Liu, Y. and West, S.C. Distinct functions of BRCA1 and BRCA2 in double-strand break repair. **Breast Cancer Res.** 4 (2002) 9-13.
35. Manke, I.A., Lowery, D.M., Nguyen, A. and Yaffe, M.B. BRCT repeats as phosphopeptide-binding modules involved in protein targeting. **Science** 302 (2003) 636-639.
36. Yu, X., Chini, C.C., He, M., Mer, G. and Chen, J. The BRCT domain is a phospho-protein binding domain. **Science** 302 (2003) 639-642.
37. Beckmann, M.W., Schnurch, H.G., Bodden-Heidrich, R., Mosny, D.S., Crombach, G., Nitz, U., Achnoula, M. and Bender, H.G. Early cancer detection programmes for women at high risk for breast and ovarian cancer: a proposal of practical guidelines. **Eur. J. Cancer Prev.** 5 (1996) 468-475.
38. Hanby, A.M., Kelsell, D.P., Potts, H.W., Gillett, C.E., Bishop, D.T., Spurr, N.K. and Barnes, D.M. Association between loss of heterozygosity of BRCA1 and BRCA2 and morphological attributes of sporadic breast cancer. **Int. J. Cancer** 88 (2000) 204-208.
39. Wright, D.K. and Manson, M.M. Sample preparation from paraffin-embedded tissues. in: **PCR Protocols: A Guide to Methods and Application,** (Innis, M.A., Ed.), Academic Press, Inc., 1990, 153-156.
40. Rolfs, A., Schuller, I., Finckh, U. and Weber-Rolfs, J. PCR: clinical diagnostic and research. Springer Lab., 1992, 81-84.
41. Cawkwell, L., Bell, S.M., Lewis, F.A., Dixon, M.F., Taylor, G.R. and Quirke, P. Rapid detection of allele loss in colorectal tumours using microsatellites and fluorescent DNA technology. **Br. J. Cancer** 67 (1993) 1262-1267.
42. Simpson, A.J. The natural somatic mutation frequency and human carcinogenesis. **Adv. Cancer Res.** 71 (1997) 209-240.

43. Thiagalingam, S., Foy, R.L., Cheng, K., Lee, H.J., Thiagalingam, A. and Ponte, J.F. Loss of heterozygosity as a predictor to map tumor suppressor genes in cancer: molecular basis of its occurrence. **Curr. Opin. Oncol.** 14 (2002) 65-72.
44. Chen, J.J., Silver, D., Cantor, S., Livingston, D.M. and Scully, R. BRCA1, BRCA2, and Rad51 operate in a common DNA damage response pathway. **Cancer Res.** 59 (1999) 1752s-1756s.
45. Dasika, G.K., Lin, S.C., Zhao, S,, Sung, P., Tomkinson, A. and Lee, E,Y. DNA damage-induced cell cycle checkpoints and DNA strand break repair in development and tumorigenesis. **Oncogene** 18 (1999) 7883-7899.
46. Wang, W.W., Spurdle, A.B., Kolachana, P., Bove, B., Modan, B., Ebbers, S.M., Suthers, G., Tucker, M.A., Kaufman, D.J., Doody, M.M., Tarone, R.E., Daly, M., Levavi, H., Pierce, H., Chetrit, A., Yechezkel, G.H., Chenevix-Trench, G., Offit, K., Godwin, A.K. and Struewing, J.P. A single nucleotide polymorphism in the 5' untranslated region of *RAD51* and risk of cancer among *BRCA1/2* mutation carriers. **Cancer Epidemiol. Biomarkers Prev.** 10 (2001) 955-960.
47. Levy-Lahad. E, Lahad, A., Eisenberg, S., Dagan, E., Paperna, T., Kasinetz, L., Catane, R., Kaufman, B., Beller, U., Renbaum, P. and Gershoni-Baruch, R. A single nucleotide polymorphism in the *RAD51* gene modifies cancer risk in *BRCA2* but not *BRCA1* carriers. **Proc. Natl. Acad. Sci. USA.** 98 (2001) 3232-3236.
48. Goode, E.L., Dunning, A.M., Kuschel, B., Healey, C.S., Day, N.E., Ponder, B.A., Easton, D.F. and Pharoah, P.P. Effect of germ-line genetic variation on breast cancer survival in a population-based study. **Cancer Res.** 62 (2002) 3052-3057.
49. Błasiak, J., Przybyłowska, K., Czechowska, A., Zadrożny, M., Pertyński, T., Rykała, J., Kołacińska, A., Morawiec, Z. and Drzewoski, J. Analysis of the G/C polymorphism in the 5'-untranslated region of the *RAD51* gene in breast cancer. **Acta Biochim. Pol.** 50 (2003) 249-253.
50. Romanowicz-Makowska, H., Smolarz, B. and Kulig, A. Germline BRCA1 mutations and G/C polymorphism in the 5'-untranslated region of the RAD51 gene in Polish women with breast cancer. **Pol. J. Pathol.** 56 (2005) 161-165.
51. Jakubowska, A., Narod, S.A., Goldgar, D.E., Mierzejewski, M., Masojc, B., Nej, K., Huzarska, J., Byrski, T., Górski, B. and Lubiński, J. Breast cancer risk reduction associated with the *RAD51* polymorphism among carriers of the *BRCA1* 5382insC mutation in Poland. **Cancer Epidemiol. Biomarkers Prev.** 12 (2003) 457-459.
52. Seedhouse, C., Faulkner, R., Ashraf, N., Das-Gupta, E. and Russell, N. Polymorphisms in genes involved in homologous recombination repair interact to increase the risk of developing acute myeloid leukemia. **Clin. Cancer Res.** 10 (2004) 2675-2680.

53. Carling, T., Imanishi, Y., Gaz, R.D. and Arnold, A. RAD51 as a candidate parathyroid tumour suppressor gene on chromosome 15q: absence of somatic mutations. **Clin. Endocrinol. (Oxf)** 51 (1999) 403-407.
54. Kato, M., Yano, K., Matsuo, F., Saito, H., Katagiri, T., Kurumizaka, H., Yoshimoto, M., Kasumi, F., Akiyama, F., Sakamoto, G., Nagawa, H., Nakamura, Y. and Miki, Y. Identification of Rad51 alteration in patients with bilateral breast cancer. **J. Hum. Genet.** 45 (2000) 133-137.
55. Wick, W., Petersen, I., Schmutzler, R.K., Wolfarth, B., Lenartz, D., Bierhoff, E., Hummerich, J., Muller, D.J., Stangl, A.P., Schramm, J., Wiestler, O.D. and von Deimling, A. Evidence for a novel tumor suppressor gene on chromosome 15 associated with progression to a metastatic stage in breast cancer. **Oncogene** 12 (1996) 973-978.
56. Gonzalez, R., Silva, J.M., Dominguez, G., Garcia, J.M., Martinez, G., Vargas, J., Provencio, M., Espana, P. and Bonilla, F. Detection of loss of heterozygosity at RAD51, RAD52, RAD54 and BRCA1 and BRCA2 loci in breast cancer: pathological correlations. **Br. J. Cancer** 81 (1999) 503-509.
57. Shen, C.Y., Yu, J.C., Lo, Y.L., Kuo, C.H., Yue, C.T., Jou, Y.S., Huang, C.S., Lung, J.C. and Wu, C.W. Genome-wide search for loss of heterozygosity using laser capture microdissected tissue of breast carcinoma: an implication for mutator phenotype and breast cancer pathogenesis. **Cancer Res.** 60 (2000) 3884-3892.
58. Balsara, B.R., Bell, D.W., Sonoda, G., De Rienzo, A., du Manoir, S., Jhanwar, S.C. and Testa, J.R. Comparative genomic hybridization and loss of heterozygosity analyses identify a common region of deletion at 15q11.1-15 in human malignant mesothelioma. **Cancer Res.** 59 (1999) 450-454.
59. De Rienzo, A., Balsara, B.R., Apostolou, S., Jhanwar, S.C. and Testa, J.R. Loss of heterozygosity analysis defines a 3-cM region of 15q commonly deleted in human malignant mesothelioma. **Oncogene** 20 (2001) 6245-6249.
60. Natrajan, R., Louhelainen, J., Williams, S., Laye, J. and Knowles, M.A. High-resolution deletion mapping of 15q13.2-q21.1 in transitional cell carcinoma of the bladder. **Cancer Res.** 63 (2003) 7657-7662.
61. Hanby, A.M., Kelsell, D.P., Potts, H.W., Gillett, C.E., Bishop, D.T., Spurr, N,K, and Barnes, D.M. Association between loss of heterozygosity of BRCA1 and BRCA2 and morphological attributes of sporadic breast cancer. **Int. J. Cancer** 88 (2000) 204-208.
62. Johnson, S.M., Shaw, J.A. and Walker, R.A. Sporadic breast cancer in young women: prevalence of loss of heterozygosity at p53, BRCA1 and BRCA2. **Int. J. Cancer** 98 (2002) 205-209.
63. Smith, S.A., Easton, D.F., Evans, D.G. and Ponder, B.A. Allele losses in the region 17q12-21 in familial breast and ovarian cancer involve the wild-type chromosome. **Nat. Genet.** 2 (1992) 128-131.
64. Garcia, J.M., Rodriguez, R., Dominguez, G., Silva, J.M., Provencio, M., Silva, J., Colmenarejo, A., Millan, I., Munoz, C., Salas, C., Coca, S.,

Espana, P. and Bonilla, F. Prognostic significance of the allelic loss of the BRCA1 gene in colorectal cancer. **Gut** 52 (2003) 1756-1763.

65. Silva, J.M., Gonzalez, R., Provencio, M., Dominguez, G., Garcia, J.M., Gallego, I., Palacios, J., Espana, P. and Bonilla, F. Loss of heterozygosity in BRCA1 and BRCA2 markers and high-grade malignancy in breast cancer. **Breast Cancer Res. Treat.** 53 (1999) 9-17.
66. Rio, P.G., Maurizis, J.C., Peffault de Latour, M., Bignon, Y.J. and Bernard-Gallon, D.J. Quantification of BRCA1 protein in sporadic breast carcinoma with or without loss of heterozygosity of the BRCA1 gene. **Int. J. Cancer** 80 (1999) 823-826.
67. Santos, S.C., Cavalli, L.R., Cavalli, I.J., Lima, R.S., Haddad, B.R. and Ribeiro, E.M. Loss of heterozygosity of the BRCA1 and FHIT genes in patients with sporadic breast cancer from Southern Brazil. **J. Clin. Pathol.** 57 (2004) 374-377.