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Loss of Heterozygosity in the *RAD54B* Region is Not Predictive for Breast Carcinomas

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This study was carried out to evaluate the loss of heterozygosity (LOH) in the 8q12–q24.1 chromosomal region, containing *RAD54B* gene in breast cancer. Polymorphic markers D8S539 and D8S543 were used. For alleles frequency estimation 100 primary breast cancers were tested. DNA was isolated from paraffin-embedded tissues and their matched blood samples. Polymerase chain reaction amplified products of normal and tumor DNA pairs were compared in ABI PRISM 377 DNA sequencer. In analyzed cases LOH was found in 1% and 2% of informative cases for microsatellite markers D8S539 and D8S543, respectively. This date indicate, that LOH isn't predictive for breast cancer.

Introduction

The *RAD52* epistasis group genes (*RAD50-57, XRS2* and *MRE11*) were identified in the yeast *Saccharomyces cerevisiae* through mutants exhibiting defects in recombination and repair of DNA double-strand breaks (DSBs) in mitosis and meiosis.The highly conserved human *RAD52* epistasis group homologues, *RAD50, RAD51, RAD52, RAD54* and *MRE11*, are implicated in mammalian DSB repair. The reduced frequency of targeted integration and hypersensitivity to ionizing radiation in RAD54 null cells have indicated that RAD54 function is conserved from *Saccharomyces cerevisiae* to mice [1, 5, 15]. In contrast, RAD51 and RAD52 functions are not conserved throughout evolution. RAD51 in higher eukaryotes is essential for cell viability, while yeast RAD51 is not [8, 12, 18]. RAD52 does not affect radiosensitivity in higher eukaryotes while inactivation of RAD52 in yeast results in hypersensitivity to ionizing radiation [13, 21].

Human *RAD54B* was isolated by screening a testis cDNA library with an expressed sequence-tagged (EST) probe homologous to *Saccharomyces cerevisiae RAD54* [6]. The gene encodes a protein containing ATPase domains that are highly conserved in members of the *SWI2/SNF2* superfamily, including *RAD54*. Consistent with its putative role in recombination, RAD54B forms a protein complex with RAD51, RAD54 and BRCA1 [16, 17, 19].

The expression of RAD54B was high in testis and spleen, which are active in meiotic and mitotic recombination. Human *RAD54B* plays a critical role in targeted integration in human cells without affecting cell growth, cell survival to DNA-damaging agents. With the exception of *RAD52*, other recombination genes, including *RAD51, RAD54, MRE11 and RAD51* paralogs, do affect the sensitivity to such agents [9, 13].

Polymorphism in multiple pathways may act synergistically with enviromental carcinogen damage to increase cancer susceptibility [12]. The loss of heterozygosity (LOH) of polymorphic genetics markers is thought to reflect the general chromosomal localizations of tumor suppressor genes in which the random loss of one allele unmasks a mutated and non-functional allele on the other chromosome, conferring a growth advantage leading to clonal expansion of cells with the alteration [9]. The aim of the work was to verify if allelic losses in the chromosomal regions of *RAD54B* gene could be notoriously found in breast cancer.

Material and Methods

Patients

A hundred paraffin-embedded tissues from patients with breast cancer and matched blood samples were obtained at the Department of Clinical Pathomorphology, Polish Mother's Memorial Hospital Research Institute, Lodz, Poland. The mean age of patients was 53, ranging from 28 to 77. Among the patients 34 were under or at the age of 50 and 66 were above the age of 50. All tumor specimens underwent clinicohistopathological evaluations. Regarding histological type 86 cases were classified as ductal and 14 cases were classified as lobular carcinoma. The series included 14 cases at stage I, 82 at stage II and 4 at stage III according to the modified Bloom-Richardson criteria; 62 positive and 38 negative cases in respect to estrogen receptors; 57 positive and 43 negative cases in respect to progesterone receptors. According to tumor size, the samples were divided into T_1 ≤ 2 cm (64 cases), T_2 2–5 cm (31 cases) and T_3 > 5 cm (5 cases) categories [4]. According to the appearance of metastasis in the axillary lymph nodes, the samples were allocated to N_0 (63 cases), N_1 (32 cases) and N_2 (4 cases) categories. In 97 studied cases there were no distal metastases (M_0) and 3 cases were classified as M_1.

DNA isolation

DNA was isolated from peripheral blood and tissue samples following standard phenol extraction procedure. The paraffin-embedded tissue samples were extracted with xylene to remove the paraffin [14, 20].

PCR conditions and primers

Two microsatellite markers D8S539 and D8S543 flanking dinucleotide CA microsatellite repeats were used to determine LOH in the 8q12-q24.1 region, containing *RAD54B* gene in breast cancer. Sequences for primers are listed in the Human Genome Database (www.gdb.org). Primers were synthesized and labeled fluorescently in Applied Biosystems (USA). The sequences of primers used for PCR are shown in Table 1. Polymerase chain reaction (PCR) was performed in 7.5 μl volumes using 50 ng of genomic DNA template, 0.3 units of AmpliTaq Gold ^{TM} DNA polymerase, 1 x GeneAmp® PCR Gold Buffer, 1 mM GeneAmp dNTP Mix, 2.5 mM magnesium chloride, 5 pmol of either forward or reverse primer end labeled with the dye phosforamidite 6-FAM and TET (Applied Biosystems, USA). 30-cycles amplification (denaturation, annealing, and extension) was done in GeneAmp 2400 PCR System (Perkin-Elmer, USA). Profile times and temperatures were: 95°C for 5 min; 30 cycles – 94°C for 15 s, 55°C for 30 s, 72°C for 30 s; 72°C for 10 min.

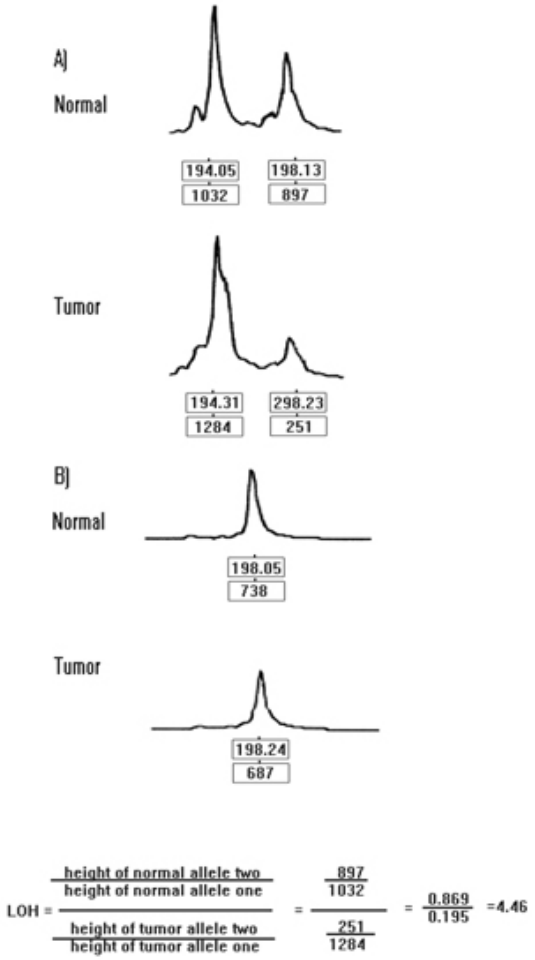

Fig. 1. Examples of informative (A) and non-informative (B) cases for microsatellite marker D8S539. LOH of the 2-allele pairs (194 base-pairs and 198 base-pairs) was defined mathematically as shown.

TABLE 1
Characterization of the microsatellite markers used

Microsatellite marker	Primer sequences (5'-3')	Dye (colour)	Size (bp)
D8S539	* CAC CTG GCA TAA GTT TCT ACT CA; ** TTC TTG CTT TCA TCA ATG TGAC	6-FAM (blue)	190–200
D8S543	* TGG TGT CAT TGC TTT CTA GTCT; ** TGC ACA GGT GAG TAA ATT TGT AA.	TET (green)	116–140

* Upstream; ** Downstream

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 of deionized formamide, two parts of GeneScan™-350 TAMRA Size Standard and one part of loading buffer (50 mg/ml blue dextran, 25 mM EDTA). Samples were denatured at 95°C for 5 min and chilled on ice. A 3 μl of each sample were loaded in the well of the gel and run for 2 hrs in ABI PRISM 377™ DNA Sequencer (Applied Biosystems, USA). The data were collected automatically. Allele sizing was determined by GeneScan version 3.1.2 and Genotyper version 2.5 softwares (Applied Biosystems, USA). A patient was considered non-informative when only one allelic band was identified in the normal DNA line (homozygosity). A patient was considered informative when two major allelic bands were identified in the normal DNA (heterozygosity). The allele ratio was calculated as (N2/N1)/(T2/T1) for the ratio height peak of normal (N) versus tumor (T) alleles. The LOH is defined as the allelic ratio above 1.5 or below 0.5. An example of a representative LOH sample is shown in Fig 1.

Results and Discussion

The LOH analysis in *RAD54B* chromosomal region was performed for two microsatellite markers D8S539 and D8S543 using DNA isolated from the tumor and corresponding peripheral blood of each of 100 patients. Genomic deletion detected by allelic loss was observed in 1% (1/74) of informative cases for microsatellite marker D8S539 and in 2% (2/80) of informative cases for microsatellite marker D8S543 (Table 2).

Breast cancer is the most frequent malignancy in women. It is also the most common cancer-related cause of death among women [11]. High prevalence and mortality of breast carcinoma underscore the necessity for solid clarification of the molecular basis of the disease. LOH in 24 regions on 16 other chromosomes has been extensively studied in breast cancer, as it constitutes one of the commonest genetic alterations in this type of cancer [10]. Identification of chromosomal regions with allelic losses is a useful method for screening genes implicated in the breast pathogenesis and, at the same time, offers the opportunity to investigate new parameters with possibly high sensitivity and specificity for use as prognostic factors [3].

TABLE 2
LOH analysis in the *RAD54B* chromosomal region in breast cancer

Microsatellite marker	Number of informative cases	Tumors with LOH (%)
D8S539	74	1 (1)
D8S543	80	2 (2)

Although there are no data concerning genetic instability in *RAD54B* in breast cancer, mutations in this gene in human tumors have been reported. Homozygous mutations of this gene were observed in primary non-Hodgkin's lymphoma and colon cancer. An A to G transition converted Asn to Ser at codon 593 was present in lymphoma and A G to T transversion converted Asp to Tyr at codon 418 was present in adenocarcinoma of the colon (Hiramoto et al. 1999). Additionally, two neutral polymorphisms were observed. An AAT to AAC substitution at codon 250 and a GAA to GAG substitution at codon 396 were found in 61% and 6% of alleles, respectively [6].

Asn at codon 593 and Asp at codon 418 are located at a conserved position in SNF2 throughout evolution from yeast to human [7]. Although the roles of conserved motifs in member of the SNF2 superfamily remain to be demonstrated, conservation of the sequences throughout evolution strongly supports the idea that mutations in these regions might affect their function. Since these gene products are presumed to play important roles in the maintenance of genome integrity, mutations in conserved domains are expected to induce genetic instability. Thus *RAD54B* is shown to be the first gene in the SNF2 superfamily that is mutated at conserved positions in human primary cancers. However, the *RAD54B* has been found to be altered in only primary colon cancer and lymphoma, and it is therefore, possible that this gene plays an active role in different recombinational pathways [2, 6].

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