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Cancer Letters 224 (2005) 185–191
CANCER Letters
www.elsevier.com/locate/canlet

Loss of heterozygosity at the BRCA1 locus in Tunisian women with sporadic breast cancer

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Received 4 August 2004; received in revised form 30 October 2004; accepted 2 November 2004

Abstract

Breast cancer in Tunisia is characterized by a much higher incidence of aggressiveness compared with Western countries. The pattern of allelic loss at the BRCA1 locus in Tunisian women with breast carcinoma has not been studied. Therefore, the aim of this present preliminary study was mainly focused on loss of heterozygosity (LOH) analysis of the BRCA1 gene to determine if this tumor suppressor gene is involved in sporadic breast carcinoma among Tunisian women. We investigate allelic losses by analyzing three microsatellite markers in the BRCA1 region, in a panel of 21 human breast tumors. D17S1322 marker had the highest frequency of LOH (59%), followed by the D17S1323 (35%), and EDH-17B (20%). Collectively out of 21 informative cases 13 (62%) showed LOH at at least one BRCA1 locus. This data provides evidence that allelic loss at BRCA1 is a frequent event in sporadic breast tumorigenesis among Tunisian women, and suggests that the BRCA1 gene might play an important role as a tumor suppressor gene.

Keywords: Loss of heterozygosity; Breast cancer; BRCA1; Tunisia

1. Introduction

Breast cancer, occurring in both hereditary and sporadic forms, is a great-problem of public health all over the world. It represents the most common malignancy among women worldwide except in Japan [1]. The incidence is geographically variable, being highest in North-Western Europe and in North America [2]. The development and progression of familial and sporadic breast cancer is based on the accumulation of genetic alterations, including events such as activation of oncogenes as well as inactivation of tumor suppressor genes (TSGs) [3], for which loss of heterozygosity (LOH), the loss of a normal functional allele at a heterozygous locus, is one mechanism of gene inactivation. BRCA1 is a putative TSG located on chromosome 17q21 and spans 100 kb of genomic DNA, which encodes a protein of 220 kDa consisting of 1863 amino acids [4,5]. It has been shown to regulate the maintenance of genome integrity, cell-cycle control, apoptosis, and DNA repair [6,7]. While links between BRCA1 mutations

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doi:10.1016/j.canlet.2004.11.001

and inherited breast cancer are well established [8,9], there is no evidence for direct mutation of the BRCA1 gene in the sporadic form of the disease [10]. However, the loss or reduction of BRCA1 protein expression has been shown to be common in sporadic breast carcinomas [11–13], indicating the involvement of this gene in sporadic forms.

LOH is the most common type of somatic alteration found in human breast tumors [14,15]. Through testing of tumor and matched normal DNA at a series of polymorphic marker loci, numerous allelic analyses of breast cancer have demonstrated high frequencies of LOH at 17q21 [16,17], the BRCA1 locus, supporting the hypothesis that this TSG plays an important role in sporadic mammary tumorigenesis and that mechanisms other than mutation, in particular LOH, might be involved in the inactivation of this gene [16,17]. However, previous reports have shown that the frequency of LOH in BRCA1 varied among different ethnic groups, and the high value is reported in Arabic-American women [18]. This finding offer new evidence for possible racial/ethnic differences with regard to genetic alterations.

Despite the lower incidence of breast cancer in Tunisia (approximately 19 new cases per 100,000 women per year) [19], Tunisian breast cancer is characterized by a much higher incidence of aggressiveness compared with Western countries [20]. In addition, the diversity of breast cancer causes may certainly vary between populations, due to differences in genetic background, ethnic differences in lifestyle, diet, and environment factors to which individuals are exposed. Therefore, it is important to evaluate breast cancer cases from Tunisia to look for possible presence of unusual changes associated with specific ethnicities. Our present research which is the first compilation of data on the involvement of LOH in the molecular pathogenesis of breast cancer in Tunisian population aimed to define the role of BRCA1 in sporadic breast cancer from an Arabic region where breast cancer is characterized by tumor aggressiveness. LOH at the BRCA1 loci was examined by three microsatellite markers (two intragenic, one flanking) in a series of sporadic breast cancer cases. In addition, association of LOH with clinicopathological parameters was examined to reveal the biological role of this potential TSG in breast cancer development.

2. Materials and methods

2.1. Patients and tissues

Twenty-one tumors from patients undergoing surgery for breast cancer were made available for this investigation. None had a family history as determined prospectively. The mean age at diagnosis of these breast cancer patients was 46 years, and the median age was 43 years (ranging from 34 to 73 years).

All samples were obtained from the tissue bank maintained by our Department of Pathology at Farhat Hached Hospital, Sousse, Tunisia. Specimens of freshly resected breast carcinomas were collected immediately after surgical excision and stored at −80 °C until subsequent analysis. In all cases, fresh specimens of normal mammary tissue were also collected and used as matching controls. All tumors underwent histological examination by a pathologist to confirm the diagnosis of adenocarcinoma, carry out the pathological staging and to evaluate percentage of tumor cells comprising these samples. The breast cancers were classified according to the WHO histological classification [21], and were graded according to the modified Scarff, Bloom and Richardson grading system (SBR) [22]. All tumors were infiltrating ductal carcinoma. Only tumor samples containing at least 70% of neoplastic cells were included in the study. To verify this condition one 10 μm-thick cryostat section from each tumor sample was stained with hematoxylin–eosin and microscopically examined by a pathologist. Estrogen receptor (ER) and progesterone receptor (PgR) expression were determined by immunohistochemistry. Clinicopathological data (histological type, stage, grade, lymph node metastasis, estrogen and progesterone receptors, and age), paraffin blocks, and slides were available for review in all tumors.

2.2. DNA extraction

Genomic DNA was isolated from fresh-frozen tumor and corresponding normal tissue samples following standard protocols [23]. Briefly, 15–30 cryostat sections (10 μm-thick) from the tumors included in the study and from matching normal samples were digested overnight at 56 °C with