Loss of heterozygocity in an intron of pros-27 K gene revealed by microsatellite analysis

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Polymorphism of a TG dinucleotide repeat identified in intron 6 of the human proteasome core particle PROS-27 K gene was studied in relation to breast cancer. We present data on the length polymorphism of this repeat in 120 individuals from Latvia and 197 individuals from Finland. A combination of PCR and fluorescent gel electrophoresis was utilized to type the polymorphism. Twelve alleles were observed, varying in length from 10 to 23 TG repeats. Similar allele frequencies were observed in Latvian and Finnish subjects, with 17 and 20 repeats being the most frequent in both populations. We suggest that this TG dinucleotide repeat could be utilized as a prospective marker for genetic linkage and association studies of common diseases.

Key words: proteasomes, PROS-27, TG repeat, intron, breast cancer

INTRODUCTION

Proteasomes (prosomes, multicatalytic proteinase (MCP) particles) are complexes of proteins with molecular weights of 20,000 to 36,000 Da and exhibiting the multicatalytic proteinase activity that is primarily involved in degrading ubiquitinated proteins [1]. Proteasome core proteins are classified into two superfamilies (alpha and beta), which have been further subdivided into 14 families on the grounds of phylogenetic analysis [2]. The ubiquitin-proteasome mechanism is involved in the degradation of oncogene and tumour suppressor products and has been implicated in the pathogenesis of cancer [1, 3]. Qualitative and quantitative changes in proteasome structure have already been observed in breast cancer and leukemias [4, 5]. Recently we have described a polymorphic TG repeat in the human proteasome core particle, PROS-27 K (IOTA, PSMA6) gene [3] named HSMS006. In the present study we investigate the frequency of different alleles and the stability of this microsatellite in breast cancer tissues.

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MATERIALS AND METHODS

Tissue specimens

In our study we used 51 paraffin sections of breast cancer tissues collected in Finland. Blood and breast cancer tissues were taken from 14 patients that underwent surgery in the Latvian Oncology Center; the blood from the same patients was collected several days after the operation.

DNA extraction

DNA from human blood and fresh breast cancer tissues was extracted using a conventional protocol of proteinase digestion and salting out. To obtain DNA from paraffine sections we used a modified method of Frank et al. [6]. A 25- μm section was placed in an eppendorf tube, 0.5 ml of xylene was added, and the tube was vortexed twice for 10 min. Then 0.5 ml of absolute ethanol was added, the tube was vortexed for 10 s and centrifuged ar 12 000 rpm for 5 min at room temperature, the supernatant was discarded, and the tube was dried on a filter paper. The step was repeated. Samples were washed twice with absolute ethanol. One millilitre ml of ethanol was added, the tubes were vortexed twice for 10 s. The pellets were dried for 15 min at 65 °C. To rehydratate the specimens, 1 ml

TE pH 8.0 (10 mM Tris-HCl pH 8.0, 1 mM EDTA) was added to each pellet, the tube was incubated for 10 min to 1h at room temperature and for 5 min at 100 °C in a dry bath. Incubation was continued overnight at 37 °C in an incubator. The next day the samples were cooled for 10–30 min on ice and spun for 5 min at 14,000 rpm at room temperature. The supernatant was removed with a pipette, 50 µl of proteinase K solution (500 µg/ml proteinase K in 10 mM Tris-HCl pH 8.0, 1 mM EDTA) was added to each sample. Digestion was performed for 3 h at 56 °C in a water bath and continued overnight at 37 °C in an incubator. Proteinase K was inactivated at 100 °C for 10 min in a hot block. Samples were centrifuged for 5 min at 12,000 rpm at room temperature. DNA quantity and integrity was determined by electrophoresis in agarose gel.

PCR and fragment analysis

The repeat was amplified from genomic DNA samples using the following primers: forward, 5'-CTAATTGACTTGCAGTTGCTGG-3' (labelled with the fluorescent dye CY5; supplied by Pharmacia) and reverse, 5'-AACTGCCTCACAGGGCTG-3'. The reaction mix contained 100 ng of genomic DNA; 1.5 mM $MgCl_2$, 40 µM of dCTP, dGTP, dTTP, dATP; 0.4 µM of each primer, 1U of Taq polymerase in 25 µl reaction mixture. PCR conditions were: 3 min at 94 °C, followed by 45 cycles of 94 °C for 1 min, 60 °C for 1 min and 72 °C for 2 min. Chain elongation was continued after the last cycle for 7 min [7]. PCR was performed on a Hybaid Express PCR machine in 96-well format. Fragment length analysis was performed on an automated laser fluorescence (ALF II Express, Pharamacia) sequencer using a short gel cassette. Denaturing polyacrylamide gels [(PAAG) (6% w/v; 0.35 mm thick)] were prepared following the manufacturer's protocol (Pharmacia). In each lane, 73 bp and 231 bp fragments amplified in the laboratory were included as standards. The sample standard mix was heat-denatured and snap-cooled before loading. The gels were run in 0.5 TBE buffer (0.045 Tris-borate, pH 8.3, and 1 mM EDTA) with 600 V, 50 mA and 50 W. The gel temperature was 70 °C. Fragment sizes were calculated using the Fragment Analyser 1.02 computer program (Pharmacia) in comparison with the internal size standards (73, 122, 196, 231 bp, prepared in the laboratory).

Statistical analysis was performed using the χ^2 criterion.

RESULTS

Comparison of the HSMS006 alleles found in breast cancer specimens collected in Finland (51 specimens) with these described in the Finnish population [8] did not reveal any novel alleles in breast cancer, indicating a relative stability of this microsatellite in the given neoplasia type (Fig. 1). However, TG15 and TG16 repeats appeared to be more frequent in breast cancer DNA as compared to the population data, and the differences were statistically significant ($p < 0.01$). This fact could indicate either eventual linkage of the shorter alleles to breast cancer, loss of heterozigosity in breast cancer cells, or certain instability of the microsatellite following malignisation. None of these hypotheses could be tested on the basis of the above data. The following studies were performed on DNA extracted from the breast cancer tissue and blood obtained from the same patient.

The data on parallel determination of the HSMS006 alleles in breast cancer tissues and blood cells are presented in Table. In most samples studied the marker alleles were identical in normal and neoplastic

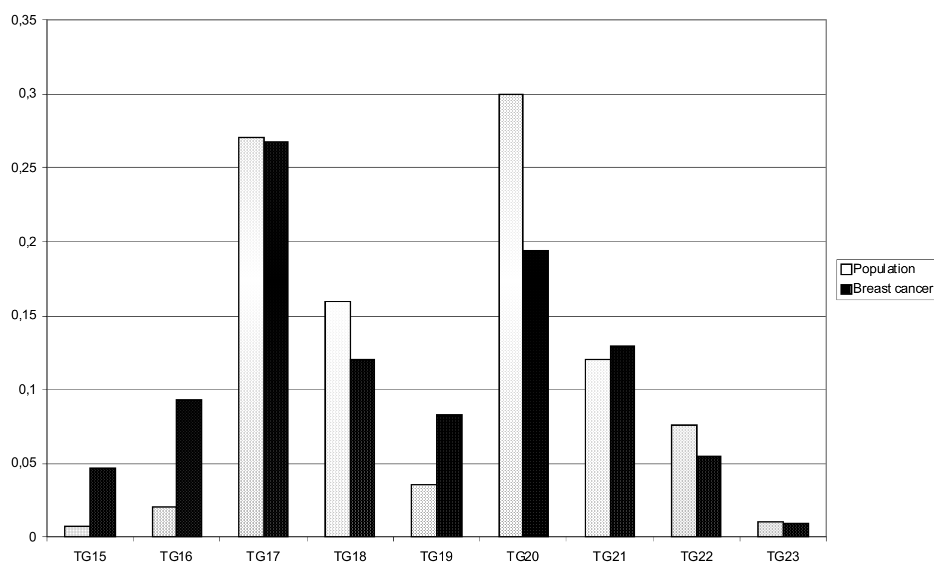

Fig. 1. Allele frequencies of TG repeat in the intron 6 of PROS-27 gene (HSMS 0006) in the Finnish population (light columns) and in breast cancer tissue samples collected in Finland (dark columns). Allele frequencies were calculated directly from the sample genotype data. Significance of differences between the groups was evaluated according to c^2 test ($p < 0.05$). Abscissa – alleles; ordinate – frequency. Differences between the groups for alleles TG15 and TG16 are statistically significant

Table. **HSMS 006 alleles in DNA extracted from breast cancer tissue and blood cells of the same patient of the Latvian Oncology Center. BC – breast cancer tissue, BCB – blood cells of a breast cancer patient**

Specimen	Genotype, TG repeats in HSMS006	Specimen	Genotype, TG repeats in HSMS006
BC-1	16/17	BC8	0/0
BCB-1	16/17	BCB8	17/21
BC-2	18/20	BC9	21/23
BCB-2	18/20	BCB9	21/23
BC3	18/23	BC10	0/0
BCB3	18/23	BCB10	17/22
BC4	21/21	BC11	21/21
BCB4	21/21	BCB11	21/21
BC5	18/23	BC12	18/18
BCB5	18/23	BCB12	18/18
BC6	21/24	BC13	20/23
BCB6	21/24	BCB13	20/23
BC7	18/18	BC14	18/18
BCB7	18/18	BCB14	18/23

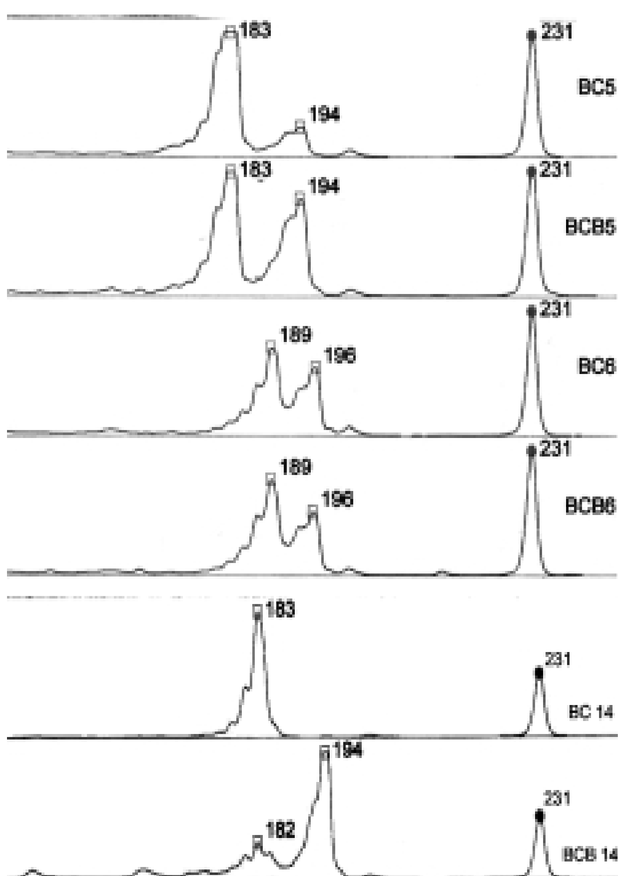

Fig. 2. Electrophoregrams of amplification products obtained using a set of primers for HSMS006 in DNA extracted from breast cancer tissue and blood cells of the same patient (patients 5, 6 and 14). BC – breast cancer tissue, BCB – blood cells of a breast cancer patient. Peak on the right – molecular weight marker (231 bp)

tissues, however, differences were observed in some patients. Figure 2 presents electrophoregrams of selected patients. For example, in patient 6 HSMS006 allele distribution in blood cell and cancer cell DNA is identical (Fig. 2). Patient 5 appears to differ in this respect: although both alleles TG18 and TG23 were observed in DNA extracted from his blood and tumor tissue, the ratios of amplification product quantities were different. Evidently the tumor DNA contains more template for the TG18 allele amplification product (183 bp in size) as compared to the TG23 allele amplification product (194 bp in size). Correspondence of the size of the amplified product to the number of the TG repeats was determined earlier [8]. This indicates loss of heterozogosity (LOH) in the tumor tissue. In patient 14 this phenomenon is even more pronounced, TG23 allele (194 bp) is completely absent from the tumor tissue, the tumor cells are TG18 heterozygotes. No amplification of HSMS006 marker was achieved from the DNA extracted from breast cancer tissue taken from patient 8, although control amplifications with other sets of markers indicated a sufficient quality and integrity of this DNA sample. Development of a mutation preventing amplification with chosen primers is possible in this case.

DISCUSSION

The presented data indicate involvement of the PROS-27 K gene intron 6 in the heterozigosity loss process coupled to breast cancer progression. Both heterozigocity loss and microsatellite instability are common in sporadic and hereditary breast cancers [9–11]. Moreover, several intronic microsatellite alleles located in distinct genes determine the risk of breast cancer development [12, 13]. Changes in the proteasome protein spectrum observed in breast cancer cells [4] could reflect changes in the gene expression coupled to alterations in genomic domains of proteasomal genes. We present the first evidence for such alterations.

The 14q allelic loss was already characterized in several tumors, it is considered that this genome region harbors some putative tumor suppressors [14]. PROS-27 K protein might be one of these suppressors, as the oncogene action is regulated via proteasomal degradation of oncogene products [3]. The limited number of tumor types and patients under study does not enable us to make a general conclusion about the role of proteasome gene polymorphism in breast cancer pathogenesis. However, our first results indicate a perspective for studies in this field.

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