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Original Paper

Frequent Allelic Losses on Chromosome 13q in Human Male Breast Carcinomas

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Loss of genetic material on chromosomes 13q and 17 has been suggested to be of importance in the initiation and progression of female breast cancer, but their involvement is less well illustrated in male breast carcinomas. The present study was designed to investigate the incidence of allelic loss and microsatellite instability for chromosomes 13q, 17p and 17q in 13 sporadic male breast carcinomas using matched normal-tumour DNA samples and seven polymorphic microsatellite markers. Genetic imbalance was found in one or more informative markers in 85% of the patients, with more frequent loss of heterozygosity and microsatellite instability at loci on chromosome 13q. Thus, a high incidence of allelic losses was observed at the retinoblastoma gene (4/6) and likewise at the *D13S263* locus (7/12), which also exhibited the highest frequency of microsatellite instability. The intragenic microsatellite in intron 1 of the *TP53* gene on chromosome 17p revealed loss of heterozygosity in 3 of 8 informative patients. The investigated proximal region of chromosome 13q is postulated to harbour several potential tumour suppressor genes associated with female breast cancer. The high incidence of allelic losses at the *D13S263* microsatellite, located distal to both the *BRCA*2 and the *Brush-1* loci but proximal to the retinoblastoma gene, possibly indicates the presence of an additional tumour suppressor gene which may be involved in male breast carcinomas. However, this hypothesis needs verification in an extended study of male breast carcinomas. © **1997 Published by Elsevier Science Ltd.**

Key words: tumour suppressor gene, male breast carcinomas, microsatellite instability, loss of heterozygosity and chromosome 13q
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INTRODUCTION

BREAST CANCER occurs rarely in men, representing approximately 1% of all breast cancer cases, and is more often advanced at diagnosis compared with female breast cancer. However, the prognosis, when corrected for stage and age, is similar to that of females. Several factors have been reported to influence the aetiology of male breast cancer, including family history, Klinefelter's syndrome, alterations in hormonal status and prior history of exposure to ionising radiation or electromagnetic fields [1].

Tumorigenesis proceeds through a series of alterations in proto-oncogenes and tumour suppressor genes resulting in the accumulation of mutations within individual cells as expressed by loss of heterozygosity (LOH) or microsatellite instability (MI). The majority of chromosomes have been shown to exhibit genetic instability in female breast cancer [2–4]. A significantly high incidence of allelic losses has been reported for chromosome 17, which harbours the putative metastasis suppressor gene, *nm23-H1*, and the proto-oncogene, *c-erbB-2*, on the long arm and the tumour suppressor gene, *TP53*, on the short arm of the chromosome. Allelic losses, although to a lesser extent, have also been reported for chromosome 13q [2, 3, 5]. The retinoblastoma tumour suppressor gene and two additional potential tumour suppressor loci, *BRCA2*, the breast cancer susceptibility gene, which has been associated with male breast cancer, and *Brush-1* are located on 13q12–14, a locus showing frequent LOH in female breast cancer [6–9].

Breast cancers with detected LOH in both chromosome 13q and 17p have more malignant histological features. An association between LOH on chromosome 17 and amplification of *c-erbB-2*, as well as high proliferative capacity, has been reported [2, 10, 11]. Furthermore, Radford and associates

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Table 1. *Microsatellite markers on chromosomes* 13*q*, 17*p and* 17*q*

Locus	Position	Polymorphism	Size (base pairs)	Reference
TP53	17p13.1	$(CA)_n$	103–135	Jones *et al.* [13]
D17S250	17q11.2–q12	$(CA)_n$	~162	Weber *et al.* [14]
nm23-H1	17q21.3	$(CA)_n$	~106	Hall *et al.* [15]
D13S260	13q12–13	$(CA)_n$	158–173	Gyapay *et al.* [16]
D13S219	13q12–13	$(CA)_n$	117–127	Gyapay *et al.* [16]
D13S263	13q12–13	$(CA)_n$	145–165	Gyapay *et al.* [16]
Rb1	13q14.2	TET	158–208	Yandell *et al.* [17]

$(CA)_n$; CA repeat. TET; tetranucleotide repeat polymorphism.

[12] performed allelotyping of ductal carcinoma *in situ* of the breast and found the highest frequency of LOH on chromosomes 8p, 13q, 16q, 17p and 17q. These findings suggest an important role for chromosomes 13q and 17 in the initiation and progression of female breast cancer. However, the pattern of genetic alterations in male breast cancer is less well investigated. The present study was designed to investigate the involvement of chromosomes 13q and 17 in sporadic male breast cancer by determining the incidence of LOH and MI.

MATERIALS AND METHODS

Patients

Male breast cancer patients, diagnosed in the South-East Sweden Health Care Region between 1980 and 1992, were included in the investigation. Formalin-fixed paraffin-embedded material was obtained from the Departments of Pathology in Linköping, Norrköping, Jönköping and Kalmar. Tumour and matched normal DNA from adjacent tissue or uninvolved lymph nodes were available in 13 patients.

Tissue and DNA samples

A microdissection technique was used in order to separate normal and tumour tissue. A 5 μm section from each paraffin block was stained with haematoxylin & eosin, and areas with entirely normal cells and areas consisting of more than 50% cancer cells were selected by a pathologist. The selected parts were separated and collected from each case by microdissection from 30 μm paraffin sections. Furthermore, the tumours were histologically graded.

PCR

The isolated DNA were examined for genetic alterations at seven different microsatellite loci (Table 1) and, in addition, mutations in exons 5–8 in the *TP53* gene were investigated. Amplification of DNA was carried out by 30–40 cycles of PCR with the following conditions: denaturation steps were performed for 1 min at 94°C except in the first cycle where the time was extended to 3 min. The annealing temperature ranged from 50 to 56°C for 1 min, and the extension steps were performed at 72°C for 1 min. The PCR mixture contained a buffer solution (200 mM ammonium sulphate, 750 mM Tris-HCl pH 9.0, 0.1% Tween 20), 2 mM $MgCl_2$, 200 μM dNTPs, 20 μM of each primer, 0.5 U Taq 1 polymerase (SDS Promega, Falkenberg, Sweden) and genomic DNA in a final reaction volume of 20 μl.

PCR–LOH analysis

Amplified PCR products from normal and tumour tissue were subjected to 10 cycles of PCR with the addition of [α-^{32}P]dATP before dilution (1:1) with loading buffer (98% (v/v) formamide, 0.1% (w/v) xylene cyanol FF, 0.1% (w/v) bromophenol blue and 10 mM EDTA). The 5 μl aliquots were then loaded on to a denaturating, preheated (45°C), 6% polyacrylamide gel containing 7 M urea and TBE buffer (45 mM Tris-borate and 1 mM EDTA). Electrophoresis was carried out at 60 W for 1–2 h before the gel was dried and exposed to X-ray film at –70°C with an intensifying screen. A deletion was scored if one allele was absent or considerably weaker upon visual inspection of tumour and normal allele intensity.

SSCA and sequence analysis of TP53

The PCR products of exons 5–8 from tumour DNA were labelled by inclusion of [α-^{32}P] dATP through 10 cycles of PCR. Five μl of 0.2% SDS/20 mM EDTA and 10 μl of loading buffer (30% (w/v) sucrose, 0.25% (w/v) bromophenol blue) were added to 5 μl of labelled PCR product. Electrophoresis was performed on a non-denaturating 6% polyacrylamide gel (45 mM Tris-borate and 1 mM EDTA) at 4 W for 14–18 h. Samples with mobility shift were selected for direct DNA sequencing using [γ-^{32}P]dATP end-labelled primers and cycle sequencing (fmol cycle sequencing kit, Promega).

Table 2. *Distribution of allelic loss (LOH) and microsatellite instability (MI) observed with seven microsatellite markers on chromosomes* 13*q*, 17*p and* 17*q and, in addition, histological grade by Elston*

		Locus						
		Chromosome 13q				Chromosome 17		
Patient	Tumour grade	*D13S260*	*D13S219*	*D13S263*	*Rb1*	*D17S250*	*nm23-H1*	*TP53*
1	II	○	—	▨	—	●	—	—
2	II	○	●	○	▩	○	○	○
3	III	▩	●	▨	●	○	●	●
4	III	○	○	○	●	○	○	○
5	II	○	○	▨ ●	▩	▩	○	▩
6	III	●	○	●	●	○	○	●
7	III	—	○	○	▩	▩	▩	○
8	III	▩	●	●	▩	○	▩	▩
9	II	▩	○	●	○	▩	○	▩
10	II	▩	▩	●	▩	▩	○	▩
11	III	▨ ●	▩	●	—	▨	○	●
12	II	○	—	—	○	○	○	○
13	III	○	—	●	●	—	○	○

○ = Normal, ● = LOH, ▨ = MI, ▩ = not informative, — = not done.

RESULTS

Genetic instability was found in one or several informative markers on chromosomes 13q or 17 in 11 of the 13 (85%) patients. Chromosome 13q revealed instability in 85% of the cases, while the loci on 17q and 17p displayed instability in 35% and 38% of the tumours, respectively. None of the 6 histological grade II tumours and 5 of 7 grade III tumours showed loss of heterozygosity in more than one polymorphic marker. Microsatellite instability was observed in 25% (3/12) of the cases in the *D13S263* locus and overall in 31% of the tumours (Table 2).

As shown in Table 2, loss of heterozygosity on chromosome 13q was most frequently found for the polymorphic marker of the retinoblastoma gene (4/6) and at the *D13S263* locus (7/12). Allelic losses were less common on chromosome 17, with the exception of the intragenic microsatellite within the *TP53* gene, which displayed LOH in 3 of the 8 informative cases. Figure 1 shows examples of allelic imbalance at the *D13S263* locus. Direct DNA sequencing revealed one silent mutation in patient 9 and a stop codon in patient 12 in exon 5 of the *TP53* gene (Figure 2).

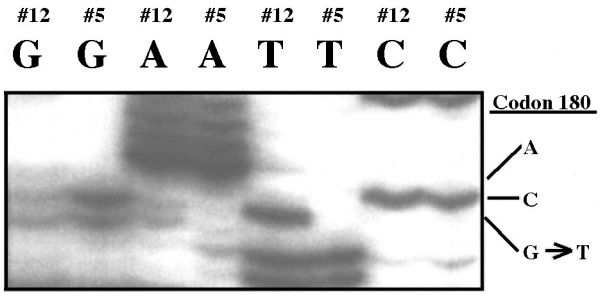

Figure 2. Nucleotide sequence analysis of DNA from sample 12, showing a point mutation in exon 5.

DISCUSSION

The investigated proximal part of chromosome 13q harbours several potential tumour suppressor loci which are involved in female breast carcinomas. We observed a peak of allelic losses at the *D13S263* locus, which is proximally flanked by the centromeric marker *D13S219* and distally by the retinoblastoma gene. LOH in these flanking alleles appeared to be independent of each other in the present study. This finding contrasts to the results of Schott and associates [6] who, in a survey of 76 female breast tumours, found that in all cases where the samples were informative for both the retinoblastoma gene and the *D13S219* locus, the results were identical. Furthermore, in the present study, LOH at the *D13S263* locus was usually different from that of the *D13S219* locus and the retinoblastoma gene. In conclusion, the high frequency of allelic losses centred around *D13S263* supports the assumption of a potential tumour suppressor gene in this region that may be involved in the development and progression of male breast cancer. However, due to the small number of cases investigated, this hypothesis needs verification in an extended study. Other loci with high frequency of LOH, which are supposed to contain tumour suppressor genes involved in male breast cancer, have been identified on chromosomes 11q13 and 8p [18, 19]. The low proportion of mutations in the tumour suppressor gene *TP53*, in the present study, contrasts with results obtained by Anelli and associates [20].

A tendency towards more frequent allelic losses was found in tumours classified as histologically high-grade malignant, suggesting that LOH may occur at a late stage in tumour progression, an observation previously made by Yee and associates [21]. The relatively low proportion of allelic loss in chromosome 17, mainly restricted to the metastasis suppressor gene, *nm23-H1*, and the *D17S250* locus, contrasts with results previously reported from female breast cancer [2, 4, 22]. Possibly, different biological features of male breast cancer, as compared with female breast cancer, may be indicated by a low incidence of LOH at the *nm23-H1* gene, as shown in the present study as well as by Sanz-Ortega and associates [19], and the relatively high proportion of LOH at the *D13S263* locus in the present study as compared with findings by Kerangueven and associates [23] in female breast

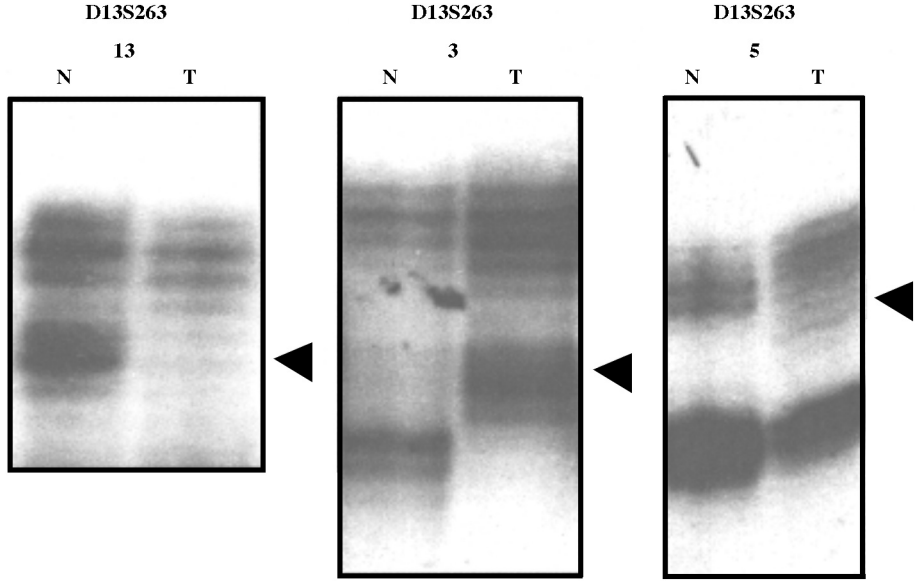

Figure 1. Examples of loss of heterozygosity and microsatellite length polymorphism analysis of marker *D13S263* of paired normal (N) and breast tumour (T) tissue. Sample 13 shows loss of heterozygosity and sample 3 expresses microsatellite instability. Both allelic loss and replication errors were found at the *D13S263* microsatellite repeat in sample 5.

cancer. Additional differences might be the low incidence of allelic losses in both the *D17S250* locus and in the *D13S260* locus, i.e. regions flanking the *BRCA1* and *BRCA2* genes, respectively. However, further studies have to be performed before male breast carcinoma is genetically characterised.

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