Identification of a 0.4 Kb deletion region in 10q26 associated with endometrial carcinoma

MARGARIDA ALVES[1,2], ISABEL CARREIRA[2,3], PAULO LIBERATO[1], SÂNCIA RAMOS[4], MANUELA MAFRA[5], ALEXANDRA S. INVERNO[1,7], ANA T. MAIA[1,8], ANA PAULA MARTINS[4], MIGUEL BRITO[6] and CAROLINO MONTEIRO[1,2]

[1]Faculdade de Farmácia Universidade de Lisboa, Av. Prof. Gama Pinto, 1649-003 Lisboa; [2]CIMAGO, Faculdade de Medicina, Universidade de Coimbra; [3]Faculdade de Medicina da Universidade de Coimbra, Laboratório de Citogenética, Instituto de Biologia Médica, 3000-354 Coimbra; [4]Hospital de Santa Cruz CHLO, Serviço de Anatomia Patológica, Avenida Prof. Reinaldo dos Santos, 2790-134 Carnaxide; [5]Hospital de São José CHLC, Serviço de Anatomia Patológica, Rua José António Serrano, 1150-199 Lisboa; [6]Escola Superior de Tecnologia da Saúde de Lisboa, Av. D. João II, Lote 4.69.01, 1990-096 Lisboa, Portugal

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Abstract. We have identified an allelic deletion common region in the q26 region of chromosome 10 in endometrial carcinomas, which has been reported previously as a potential target of genetic alterations related to this neoplasia. An allelotyping analysis of 19 pairs of tumoral and non-tumoral samples was accomplished using seven microsatellite polymorphic markers mapping in the 10q26 chromosomal region. Loss of heterozygosity for one or more loci was detected in 29% of the endometrial carcinoma samples. The observed pattern of loss enabled the identification of a 3.5 Mb common deleted region located between the D10S587 and D10S186 markers. An additional result from an endometrial sample with evidence of a RER phenotype may suggest a more centromeric region of loss within the above-mentioned interval. This 401.84 Kb interval flanked by the D10S587 and D10S216 markers may be a plausible location for a putative suppressor gene involved in early stage endometrial carcinogenesis.

Introduction

Uterine or endometrial cancer (EC) is the most common gynaecological cancer and the seventh most common malignant disorder worldwide. Its peak incidence occurs in the post-menopausal age group, being ten times higher in North America and Europe than in less developed countries. In 2001, EC accounted for 6% of all female cancers in the USA and incidence is rising as life expectancy increases (1,2).

Inactivation of tumor suppressor genes (TSGs) is one of the most critical steps leading to the development of cancer. Alterations in TSGs usually involve an inactivating event at one allele and the deletion of the remaining, together with deletion of the flanking DNA regions (3). At the molecular level, these deletions can be detected by loss of heterozygosity (LOH) studies using polymorphic genetic markers. In endometrial carcinoma, allelic deletions resulting in LOH have been reported for multiple chromosomal arms (4,5). Losses on chromosome 10 have been described, mainly in regions q23 and q25-q26 (6-9). Located on q23.3, PTEN, phosphatase and tensin homolog, is the most frequently altered tumor suppressor gene in endometrial carcinoma. Between 25 and 83% of endometrioid carcinomas are characterized by the presence of PTEN abnormalities, mainly due to mutation and/or allelic loss (10-12). In EC patients, mapping studies, within the 10q25-q26 region, suggest the presence of more than one TSG possibly involved in endometrial tumorigenesis (7,13). This region has also been associated with gliomas (14,15), prostate cancer (16,17) and melanomas (18,19). Mapping allelic losses on this specific chromosomal region may allow us to identify the involved TSGs in endometrial carcinoma as well as in other cancers.

In a tumor subset, the observation of new microsatellite alleles, absent in matching non-tumoral DNA, has led to the discovery of the so-called microsatellite instability event (20,21). As a marker for the replication error phenotype (RER), microsatellite instability (MSI) has been commonly associated with endometrial carcinoma (22). In sporadic endometrial carcinoma, the MSI frequency varies, ranging from 15 to 45% (10,21,23).

In an attempt to localize a minimal critical region of loss on 10q26, which could include a TSG involved in endometrial

Correspondence to: Dr Carolino Monteiro, Faculdade de Farmácia - UL. Laboratório de Genética Humana. Av. Prof. Gama Pinto, 1649-003 Lisboa, Portugal
E-mail: cm@ff.ul.pt

Present addresses: [7]INFARMED, I.P., Parque de Saúde de Lisboa, Av. do Brasil 53, 1749-004 Lisboa, Portugal; [8]Cancer Research UK Cambridge Research Institute and the Department of Oncology, University of Cambridge, Cambridge CB2 0RE, UK

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tumorigenesis, we performed an allelotyping analysis of 19 pairs of tumoral and non-tumoral DNA samples, using seven microsatellite dinucleotide polymorphic markers, mapped to the above-mentioned interval. As a complement, the studied tumors have been screened for the RER phenotype.

Materials and methods

Tissue samples and DNA extraction. Endometrial carcinoma samples from 19 patients were obtained from Santa Cruz Hospital, S. José Hospital and Magalhães Coutinho Hospital, all located in the Lisbon area. The 19 pairs of tumoral- and corresponding non-tumoral tissue were paraffin-embedded. The non-tumoral samples were non-neoplastic tissue adjacent to the tumor and have all been histopathologically analysed for the presence of infiltrating tumor cells. Specimens consisted in adenocarcinomas classified according to the World Health Organization (WHO) and staged according to the International Federation of Gynecology and Obstetrics (FIGO) system; ranging from stage IA (tumor limited to the endometrium) to IIIA (tumor invades serosa and/or adnexa and/or positive peritoneal cytology) and from grade 1 (well-differentiated type) to grade 3 (poorly differentiated type). Following mechanical disruption of tissue specimens, DNA extraction was accomplished by standard SDS/proteinase-K digestion and phenol/chloroform/isoamylic alcohol procedure (37). DNA isolation of some samples was accomplished with the QIAamp Tissue kit (Qiagen).

Microsatellite markers and PCR. For the allelotyping analysis of endometrial carcinoma samples, seven informative dinucleotide markers, spanning, ~14.5 Mb at the 10q26 chromosomal band: cen-D10S1236-D10S587-D10S216-D10S575-D10S186-D10S217-D10S212-tel were used. Information about the markers and primer sequences was obtained from public databases [Genome Database (http://www.gdb.org) and Ensembl Genome Browser (http://www.ensembl.org/Homo_sapiens/)]. PCR reactions were optimized over a range of 1.0-2.0 mM $MgCl_2$ and consisted of the following, for a total volume of 20 μl: Buffer II 1X (50 mM KCl; 10 mM Tris-HCl, pH 8.3; Applied Biosystems) at the appropriate $MgCl_2$ concentration, 200 mM dNTPs, 10 pmol of each primer, 0.5 U AmpliTaq Gold DNA Polymerase (Applied Biosystems) and 100-200 ng genomic DNA. One primer of each pair was fluorescence 5'-labelled with fluorochrome FAM, TET or HEX. PCR reactions were performed on a Perkin Elmer 9600 GenAmp PCR System (Applied Biosystems), according to the following cycling conditions: 20 sec at 94˚C, 30 sec at 55˚C and 40 sec at 72˚C for 30 cycles, finalized by a 10 min extension step at 72˚C. In order to rule out potential DNA contamination, negative controls were included in every PCR reaction set. Fluorescent PCR products were loaded on 6% polyacrylamide-8M urea denaturing gels and separated in an ABI Prism 377 DNA automated sequencer (Applied Bioystems). The automatically collected data was analyzed with Genescan software (Applied Biosystems). For result confirmation, PCR products were electrophoresed at least twice.

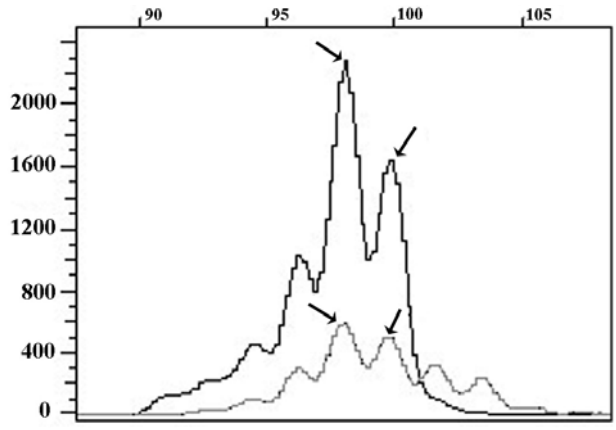

Figure 1. Example of microsatellite instability in endometrial carcinoma. Novel alleles are present in the tumour sample but not in the matched non-tumoral tissue. Arrows indicate constitutional alleles. The smaller peak on the left hand side of the main peaks consists of a stutter band.

Allelotyping analysis. For a given marker, samples with two different-sized alleles were defined as heterozygous, while those exhibiting one single allele were considered homozygous; only heterozygous patients were regarded as informative. Ratio comparison between tumoral (T) and non-tumoral (N) matched tissue was accomplished using the (T1/T2)/(N1/N2) formula, where T1 and N1 are the peak heights of the smaller allele and T2 and N2 the peak heights of the larger allele, respectively (24). For an allelic ratio <0.67 or >1.5 a sample was scored as showing LOH (25). MSI was defined by the presence of altered and/or additional alleles in the tumor DNA PCR-amplified product, as compared with matched non-tumoral DNA. In case MSI was present in at least two of the seven tested *loci*, the RER-positive phenotype was attributed.

Results

Nineteen endometrial cancers were analysed for evidence of LOH and MSI using fluorescence-based allelotyping with seven (CA) repeated polymorphic markers spanning the 10q26 region.

Allelic loss and MSI were determined by comparing tumoral- and non-tumoral DNA microsatellite allele profiles. In two of the studied samples (numbers 5 and 27), and for most of the analysed loci, we found the characteristic MSI pattern, with additional alleles present in tumor DNA but not in matched non-tumoral DNA (Fig. 1). Those samples were considered as having a RER phenotype and, therefore, have not been screened for LOH. At one locus, one sample, exhibiting heterozygosity retention, has also showed MSI.

A total of 5 out of 17 tumors (29%) demonstrated allelic loss for at least one of the l0q26 analysed loci. Of these, two tumors (40%) showed LOH for all the informative markers (samples no. 1 and 29), suggesting a total deletion of the chromosomal region under study. The three remaining tumors (60%) showed regional chromosomal losses with retention of heterozygosity for some of the markers. Fig. 2 shows all cases with at least one allelic deletion. Examples of allelic loss and heterozygosity retention are shown in Fig. 3.

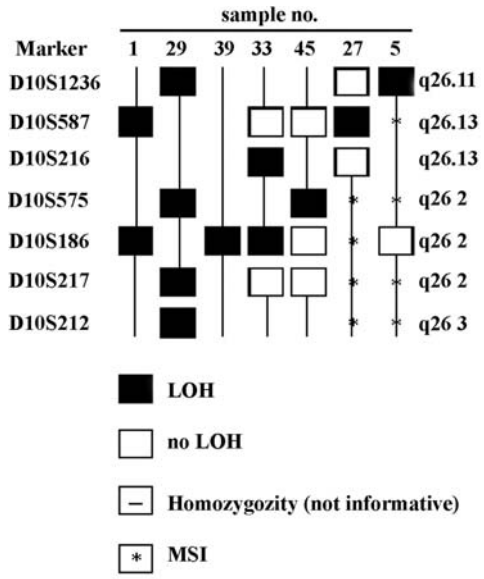

Figure 2. Allelic deletion map for seven 10q26 microsatellite markers in 7 out of 19 endometrial carcinoma samples that showed LOH at one or more marker loci. Markers have been arranged from centromere to telomere. Physical map positions and chromosomal distances between markers are shown on the right. Black boxes indicate LOH. Empty white boxes indicate heterozygosity retention. Uninformative cases are vertical line extensions. Asterisks indicate microsatellite instability.

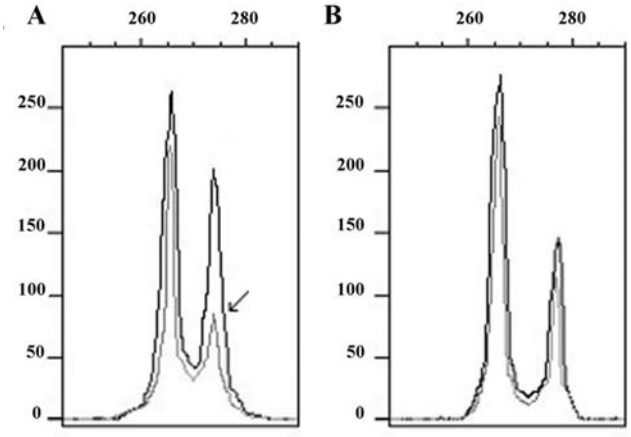

Figure 3. (A) Example of LOH. When compared with non-tumoral tissue, the smaller allele has been lost in the tumour sample (indicated by an arrow). The residual signal, depicted on the lost allele, may be due to infiltrating non-neoplastic cells. (B) Example of heterozygosity retention. Tumoral and non-tumoral tissues show the same allele profile, with no evidence of allelic loss.

Tumor samples with partial allelic deletions, corresponding to sample numbers 33 and 45 enabled us to define a common region of loss, on which a TSG may be located. Tumor no. 33 enabled the characterization of a 4.25 Mb smallest region of deletion, located between markers D10S587 and Dl0S217, cytogenetically mapped on l0q26.13 and 10q26.2, respectively. Sample no. 45 delimited a 3.50 Mb minimal region of deletion flanked by markers D10S587 and D10S186. Sample no. 27 showed retention of heterozygosity for marker D10S216 (Fig. 3b); therefore, if we include it in the LOH analysis, the region of loss is shortened to 401.84 Kb, being delimited by markers D10S587 and D10S216.

According to their histological grade and stage, tumors have also been evaluated for LOH and MSI but no correlation has been observed. It is, however, important to highlight the detection of allelic losses in early stage development tumors (sample numbers no. 1, 3 and 39).

Discussion

Previous cytogenetic and molecular genetic studies have described the involvement of chromosome 10 in endometrial carcinoma and identified the l0q23-q26 interval as being the most plausible region to contain a tumor suppressor gene involved in this type of cancer (6-9). For all endometrial samples that showed evidence of loss in the 10q26 region (5/17-29%), we were able to detect a common deleted region spanning 3.5 Mb, located between markers D10S587 and D10S186. Considering the results obtained with sample no. 27 (a RER sample which showed MSI for five out of the seven analysed loci), which clearly exhibits heterozygosity retention for marker D10S216 (Fig. 3b), that region may further be narrowed to 401.84 Kb. These results suggest that the region flanked by markers D10S587 and D10S216 may harbour a TSG involved in endometrial carcinoma tumorigenesis.

Several molecular studies have lead to the identification of genes putatively involved in various types of cancer mapped in the l0q23-q26 interval. In prostate cancer *MXI1*, located on l0q24-q25, has been lost and/or mutated (17,26). *DMBT1*, on 10q25.3-26.1, has been deleted in brain tumors and frequently appears inactivated in digestive tract, lung, breast and epithelial cancers (27-32). *MMAC1/PTEN*, on 10q23, appears mutated in endometrial, brain, prostate, bladder and breast cancers (10-12,33-37). All these genes are located in chromo-somal regions different from the one defined in the present study, suggesting a different TSG which may also be involved in endometrial tumorigenesis. The 401.84 Kb region delimited in this study overlaps that located between markers D10S587 and D10S216, previously identified in glioma by Rasheed *et al* (15). Our results confirm the importance of a 10q26 chromosomal region in cancer, which may contain, at least, one tumor suppressor gene involved in the development of endometrial carcinoma, as well as of other malignancies.

Previous studies have reported MSI as being a relative common genetic event in both sporadic and inherited forms of endometrial carcinoma (10,20). In the present study, we found MSI in 15.8% (3/19) of the studied tumors.

Supposing endometrial tumors of higher grades and stages of malignancy could result from the accumulation of genetic alterations through a development pathway, some of these alterations could also be expected to occur in early stage and lower grade endometrial tumors. Therefore, the identification in early stage tumor samples of allelic losses in the region here described, suggests that this event may consist in an early genetic pathway modification leading to endometrial carcinogenesis.

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