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ORIGINAL PAPER

# Molecular genetic defects in endometrial carcinomas: microsatellite instability, *PTEN* and beta-catenin (*CTNNB1*) genes mutations

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**Abstract**
*Purpose* The present study aims to assess the incidence of microsatellite instability (MSI) and mutations in the *PTEN* and beta-catenin (*CTNNB1*) genes in endometrial carcinomas and to analyze the detected defects in these factors in relation to each other and to the clinico-pathological features of tumors.
*Materials and methods* In a series of 56 endometrioid endometrial carcinomas, the status of MSI was determined using nine polymorphic markers, and mutations in all exons of the *PTEN* gene and in exon 3 of the *CTNNB1* gene were evaluated by SSCP and sequencing methods.
*Results* Microsatellite instability was found in 18 carcinomas (32.1%, MSI+); the remaining 38 tumors were microsatellite stable (MSI−). In 15 cases (26.8%), a loss of heterozygosity (LOH) at the studied microsatellite markers also occurred. In 29 carcinomas (51.8%), mutations were found in the *PTEN* gene and in nine tumors (16.1%) in the *CTNNB1* gene. *PTEN* mutations occurred significantly more frequently in MSI+ than in MSI− tumors (77.8 vs. 39.5%, $p = 0.007$), but, except for one, none of them was attributable to MSI. In contrast, incidence of *CTNNB1* mutations in MSI+ and MSI− tumors no significantly differed between themselves (16.7 vs. 15.8%, $p = 0.760$). Interestingly, mutations in the *CTNNB1* gene most frequently coexisted with mutations in the *PTEN* gene (7/9, 77.8%). However, this finding requires future verification on a larger group of cases. The incidence of MSI and *PTEN*, but not *CTNNB1* mutations, was significantly more common in poorly, than in well-to-moderately, differentiated tumors (G3 vs. G1 + G2; $p = 0.042$, 0.039 and 0.958, respectively).
*Conclusion* We conclude that most frequently occurring mutations in the *PTEN* gene may be a key event for the tumorigenesis of endometrioid endometrial carcinomas, while coexistence or absence of microsatellite instability or mutations in the *CTNNB1* gene may reflect the heterogeneity of molecular mechanisms contributing to the development of these tumors.

**Keywords** *PTEN* gene mutation · Beta-Catenin (*CTNNB1*) gene mutation · Microsatellite instability (MSI) · Endometrial carcinoma

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## Introduction

Two basic clinico-histopathological types of sporadic endometrial carcinoma are distinguished—type I:

endometrioid, which include the most common, rarely aggressive, estrogen-dependent adenocarcinomas; type II: nonendometrioid, which include far less frequent, estrogen-independent, more aggressive serous and clear cell tumors. Those tumors differ between themselves in both course of disease and susceptibility to treatment (Bockman 1983).

Molecular studies have confirmed the existence of two different mechanisms of carcinogenesis of sporadic endometrial carcinoma (Sherman 2000; Koul et al. 2002; Risinger et al. 2003; Lax 2004). One of them occurs in type II carcinomas and manifests itself in the first place with mutations in the *TP53* gene and overexpression of the p53 protein (Tashiro et al. 1997; Sakuragi et al. 2001). The second mechanism, associated with type I carcinomas, is characterized by a greater variability of genetic disorders. Some of them include mutations in the *KRAS*, *PTEN*, mismatch repair genes (MMR), microsatellite instability (MSI) and loss of heterozygosity in various chromosomes (LOH) and amplification and/or over-expression of *CMYC*, *INT2* and *ERBB2* oncogenes (Risinger et al. 1998; Lax et al. 2000; Konopka et al. 2004).

Mutations in the *PTEN* tumor-suppressor gene are best documented among genetic defects identified as yet in sporadic endometrial carcinomas (Kong et al. 1997; Risinger et al. 1998; Salvesen et al. 2004). *PTEN* encodes dual protein–lipid phosphate that acts on both polypeptide and phosphoinositide substrates to regulate many important processes such as cell proliferation, migration and invasion and is a suppressor of tumor growth (Tamura et al. 1999; Sansal et al. 2004). It has been reported that somatic genetic inactivation of the *PTEN* gene occurred as an early event in endometrial carcinogenesis. *PTEN* mutations have been shown to occur in about 25% of endometrial hyperplasias and in up to 80% of endometrioid endometrial carcinomas (Mutter et al. 2000). In our earlier studies, we demonstrated the presence of mutations in the *PTEN* gene in nearly 50% of endometrial carcinomas (Konopka et al. 2002, 2004). However, almost half of them had no mutation in the *PTEN* gene, which suggested that other genes are involved in their pathogenesis.

The mutations in beta-catenin (encoded by the *CTNNB1* gene) have been recently implicated in the development of some endometrial carcinomas (Mirabelli-Primdahl et al. 1999; Machin et al. 2002). Beta-catenin is included in a group of adhesive proteins and plays the role of a cell–cell adhesion regulator and also acts as a downstream component of Wnt signaling pathway and regulator of genes' transcription. The mutational activation of beta-catenin, leading to increased cell proliferation, plays a crucial role in the development of various malignancies (Bullions and Levine 1998). It seems that a mutual balance of the numerous functions of beta-catenin may be also extremely important for the normal control of growth and proliferation of endometrial cells. It has been demonstrated recently that *PTEN* regulates the localization of the free beta-catenin protein in prostate carcinoma cells (Stambolic 2002), suggesting that both these events may cooperate with each other in prostate carcinogenesis. To the best of our knowledge, only one previous study evaluated the relation of *PTEN* and beta-catenin abnormalities in endometrial carcinoma and they failed to find similar relations in this neoplasm (Wappenschmidt et al. 2004).

Microsatellite instability (MSI) is a common finding in sporadic endometrial carcinomas (Tibiletti et al. 1999; Fiumicino et al. 2001; MacDonald et al. 2000). However, molecular genetic targets for MSI in these diseases have not been fully elucidated. Some studies demonstrated a significant relation between the occurrence of MSI in endometrial carcinomas and mutations in the *PTEN* gene (Kong et al. 1997; Bussaglia et al. 2000; Baldinu et al. 2002), but the actual molecular mechanism of these relations and consequences for the endometrial carcinogenesis still remain poorly understood.

Therefore, in this study we assessed the frequency of incidence of microsatellite instability and mutations in the *PTEN* and *CTNNB1* genes in a series of endometrioid endometrial carcinomas and then analyzed the detected defects in these factors in relation to each other and to clinico-pathological features of tumors. We expect that the results of our study would allow a better understanding and insight into the mechanisms of carcinogenesis of these neoplasms.

## Materials and methods

### Patients and samples

The studied material comprised samples of 56 endometrial tumors and peripheral blood obtained from the same women who underwent surgery for endometrial carcinoma during the years 2002–2004 in the Gynecological Oncology Department of Maria Sklodowska-Curie Memorial Cancer Center of Warsaw, Poland and in the 2nd Clinic of Gynecology of Medical Academy of Wroclaw, Poland. The age range of patients of our series was 29–80 years (median age was 60.5 years). All the tumors classified according to the WHO staging system (Scully et al. 1994) were endometrioid type endometrial carcinomas. Morphological differentiation of carcinomas was as follows: 19 were regarded as G1, 28 as G2, and 9 as

G3. Staging was defined according to the International Federation of Gynecology and Obstetrics classification (FIGO) (Creasman 1989). In 10 cases, the tumor was limited to the endometrium only (FIGO stage Ia); in 31 cases, infiltration was found of the myometrium (FIGO stages Ib and Ic). In eight cases, the tumor extended to the cervix (FIGO stages IIa and IIb) and in seven cases, it extended beyond the uterus (FIGO stages IIIa–IVa). All of the analyzed cases were reviewed on diagnosis by a gynecological pathologist.

Tumors obtained during surgical procedures and peripheral blood samples of the same women were stored at −70°C. Genomic DNA was isolated by standard procedure (Sambrook et al. 1989) and was used for the PCR reaction.

### Detection of the *PTEN* gene mutations

*PTEN* mutation analysis of all nine coding exons with flanking intronic sequences was performed using polymerase chain reaction (PCR), single-strand conformation polymorphism (SSCP) and sequencing methods as we described previously (Konopka et al. 2002).

### Detection of the beta-catenin (*CTNNB1*) gene mutations

DNA fragments containing exon 3 of the *CTNNB1* gene, encoding critical phosphorylation sites, were amplified by polymerase chain reaction (preliminary PCR) using primers including 229 base pairs fragment: 5′-GATTTGATGGAGTTGGACATGGC-3′ (forward) and 5′-CCAGCTACTTGTTCTTGAGTGA AGG-3′ (reverse). PCR amplification was performed in 25 µl total volume that contained: 200 ng of genomic DNA, commercial Applied Biosystems, Foster City, CA, USA (AB) buffer (one time Tris/HCI, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, triphosphodeoxynucleotides (dNTP) of 200 µM, 1 unit Ampli Tag Gold polymerase (AB), and primers 0.5 µl each. The reaction mixture was denatured at 96°C for 10 min and subjected to 30 polymerization cycles (96°C for 30 s, 55°C for 30 s, 72°C for 30 s; final extension 72°C for 7 min). The PCR reaction was run in Gene Amp 9600 thermocycler (AB). The PCR products were subjected to SSCP analysis and samples with band shifts detected were sequenced, as we described previously for *PTEN* (Konopka et al. 2002).

### Detection of MSI and LOH

Genomic tumor DNA sample and normal peripheral blood lymphocytes DNA pairs for each case were PCR-amplified using nine microsatellite markers containing dinucleotide or mononucleotide repeat sequences (CA repeats: D2S123, D3S966, D5S346, D17S855, D17S1323, D17S250, D18S58, and single poly(A) repeats: BAT-26 and BAT-25, respectively). All primers sequences were as reported in Genome Data Base (GDB, at: http://www.gdb.org). The reactions for PCR amplification were carried out in a 25 µl total volume that contained: 200 ng of genomic DNA, commercial Applied Biosystems (AB) buffer (one time Tris/HCI, pH 8.4; 50 mM KCl), 1.5 mM $MgCl_2$, triphosphodeoxynucleotides (dNTP) of 200 µM, 1 unit Ampli Tag Gold polymerase (AB), and primers (one primer of each pair was labeled with fluorescent dye 6-FAM at the 5′ end) of 0.5 µM each. The reaction mixture was denatured at 96°C for 10 min and subjected to 30 polymerization cycles (96°C for 30 s, 50 or 60°C for 30 s, 72°C for 30 s; final extension 72°C for 10 min). The PCR reaction was run in Gene Amp 9600 thermocycler (AB).

### Separation and analysis of PCR products

The PCR products were electrophoresed through 5% denaturating polyacrylamide gel in a 377 automated DNA sequencer (AB). The PCR reaction mixture (1.5 µl of each) was mixed with 3.5 µl loading solution (2.5 µl formamide and 5 µl Blue Dextran/EDTA and 5 µl of a fluorescent-labeled, defined size marker-GS500 TAMRA as internal control). This mix was denatured for 10 min at 95°C, cooled on ice, after which 2 µl was loaded into each well on the prewarmed gel and run for 2 h at 3 kV and 51°C. While the samples were undergoing electrophoresis, fluorescence was detected in the laser-scanning region. Data were collected and automatically analyzed by the Gene Scan Analysis (AB) program. Each fluorescent peak was quantities in terms of size (in base pairs), peak height and peak area. Normal and tumor DNA pairs were compared for changes in size and height of the allele peaks of each microsatellite marker. Tumors that had instability (novel allele peaks) in at least two of the eight marked analyzed, when compared with normal DNA, were defined as unstable or microsatellite positive (MSI+). Tumors with no instability or with instability in only one marker were classified as microsatellite stable (MSI−). LOH was defined by absence or at least 50% reduction of peak height of one allele in the tumor sample after comparison with the heterozygous normal sample. Representative examples are presented in Fig. 1.

### Statistical analysis

The statistical analysis of the results was carried out using $\chi^2$-test (Yates' correction). A $p$-value of less than

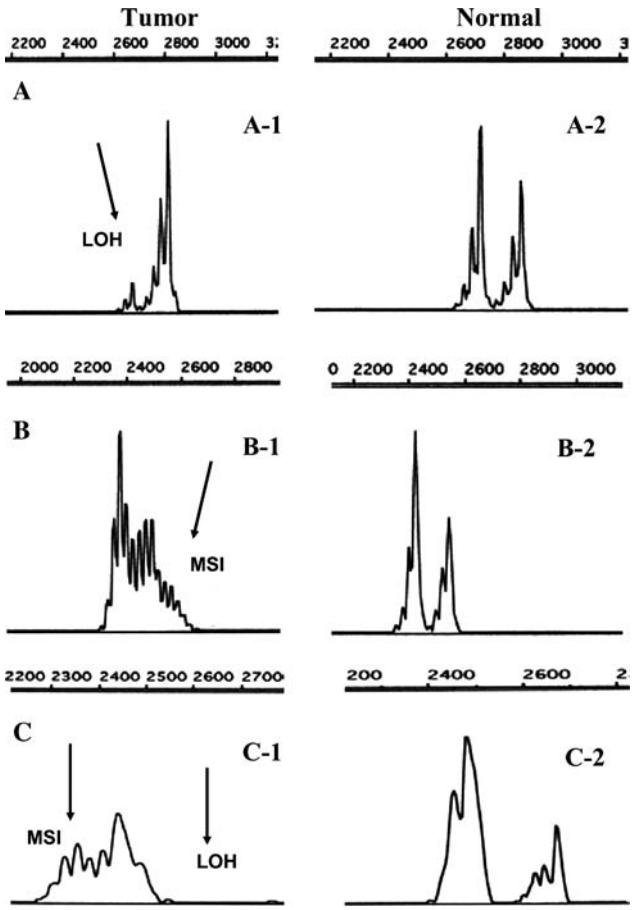


**Fig. 1** Examples of changes in allelic pattern, indicated by *arrows*, at microsatellite markers observed in endometrial carcinomas' DNA as compared to normal peripheral blood lymphocytes' DNA: **A** LOH at D17S1323, **B** MSI at D17S855 and **C** MSI and LOH at D2S123. For each microsatellite marker, the *left panel* shows tumor samples (A-1, B-1 and C-1), and the *right panel* shows the corresponding normal samples (A-2, B-2 and C-2)

0.05 was considered significant. Analysis was performed using statistical program SYSTEM MEDISTAT Ver.1.0.

## Results

### Microsatellite instability

The incidence of MSI was examined in 56 endometrioid endometrial carcinomas by comparing the alleles of nine microsatellite sequences (the markers corresponding to them were, respectively: D2S123, D3S966, D17S855, D17S1323, D17S250, D18S58, D18S346, BAT-26 and BAT-25) derived from DNA of the tumors with the alleles of analogous sequences obtained from DNA of normal blood lymphocytes of the same patient. We found that 32.1% of the tumors (18 of 56) exhibited additional alleles in two or more markers and consequently they were included into the group characterized by microsatellite instability (MSI+, Table 1). In eight tumors, changed alleles were present only in one microsatellite marker. We regarded such instability status as insufficient to qualify these cases into the group of MSI+ carcinomas. Therefore, we included them into the group of 30 remaining cases in which we found no allele alterations in any of the microsatellite markers studied. In all, 38 cases (67.9%) were numbered among the carcinomas characterized by stable DNA (MSI−). Microsatellite instability was found most frequently in the following markers: D17S1323, D3S966, D2S123 and D17S250 and less frequently in BAT-25, D17S855, D18S58 and D5S346. On the other hand, BAT-26 marker was stable in all studied cases. The frequency of MSI at each of marker is shown in Table 1.

### Loss of heterozygosity (LOH)

In the studied microsatellite markers, 22 cases of LOH were found in 15 of 56 carcinomas (26.8%); in five tumors LOH occurred simultaneously in two or three different markers. LOH was found in the following markers: D2S123, D3S966, D17S855, D17S1323, D5S346 and BAT-25. On the other hand, LOH was not detected in D17S250, D18S58 and BAT-26 markers. In 7 of 14 carcinomas LOH was accompanied by MSI, and in four cases LOH and MSI occurred simultaneously in the same markers. The described results are presented in Table 2.

### Mutations in the *PTEN* gene

In 29 out of 56 carcinomas (51.8%), 35 mutations in the *PTEN* gene were identified; six tumors had two different mutations. In Table 3, the spectrum of mutations in the *PTEN* gene occurring in MSI+ and MSI− carcinomas is compared. Most *PTEN* mutations (21/35; 60%) led to the synthesis termination of the encoded protein. The remaining group (14/35; 40%) consisted of missense mutations leading to a change of amino acids in the encoded protein. The two types of mutations mentioned occurred with a similar frequency in MSI+ and MSI− tumors (61.1 vs. 58.8% and 38.9 vs. 41.2%, respectively). *PTEN* mutations varied widely in their quality, but some of them repeated irrespective of the MSI status of the studied carcinomas. The missense mutation C388G in codon 130 (exon 5), causing a change of Arg130 to Gly, occurred in six tumors: in three cases of MSI+ and

**Table 1** Frequency of microsatellite instability (MSI) in endometrial carcinoma

| Tumor No. | Microsatellite markers[a] | | | | | | | | | No. of markers with instability | MSI % of total ($n$ = 56) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | D17S1323 | D3S966 | **D2S123** | **D17S250** | **BAT-25** | D17S855 | D18S58 | **D5S346** | **BAT-26** | | |
| 1 | − | + | + | + | + | + | + | + | − | 7 | MSI+ 32.1% |
| 2 | + | + | + | + | − | + | + | − | − | 6 | |
| 3 | + | + | − | + | + | + | − | + | − | 6 | |
| 4 | + | + | + | + | + | − | + | − | − | 6 | |
| 5 | + | + | + | − | + | + | + | − | − | 6 | |
| 6 | + | + | − | + | + | + | + | − | − | 6 | |
| 7 | + | + | + | − | + | + | − | + | − | 6 | |
| 8 | + | + | + | + | − | − | + | − | − | 5 | |
| 9 | + | − | + | + | − | − | + | + | − | 5 | |
| 10 | + | − | + | + | + | − | − | + | − | 5 | |
| 11 | + | − | + | + | + | − | + | − | − | 5 | |
| 12 | + | − | − | + | − | − | − | + | − | 3 | |
| 13 | − | − | + | − | − | − | + | + | − | 3 | |
| 14 | − | − | + | + | − | + | − | − | − | 3 | |
| 15 | + | − | − | + | + | − | − | − | − | 3 | |
| 16 | + | − | + | − | + | − | − | − | − | 3 | |
| 17 | − | + | − | − | − | + | − | − | − | 2 | |
| 18 | + | + | − | − | − | − | − | − | − | 2 | |
| 19 | − | − | + | − | − | − | − | − | − | 1 | MSI− 67.9% |
| 20 | − | + | − | − | − | − | − | − | − | 1 | |
| 21 | − | + | − | − | − | − | − | − | − | 1 | |
| 22 | − | + | − | − | − | − | − | − | − | 1 | |
| 23 | − | + | − | − | − | − | − | − | − | 1 | |
| 24 | − | − | − | − | − | + | − | − | − | 1 | |
| 25 | + | − | − | − | − | − | − | − | − | 1 | |
| 26 | − | − | − | + | − | − | − | − | − | 1 | |
| 27 | − | − | − | − | − | − | − | − | − | 0 | |
| ↓ | | | | | | | | | | ↓ | |
| 56 | − | − | − | − | − | − | − | − | − | 0 | |
| No. of tumors with instability % of total ($n$ = 56) | 15 (26.8%) | 14 (25.0%) | 13 (23.2%) | 13 (23.2%) | 10 (17.8%) | 9 (16.1%) | 9 (16.1%) | 7 (12.5%) | 0 (0%) | | |
| No. of tumors with instability % of MSI+ tumors ($n$ = 18) | 14 (77.8%) | 10 (55.6%) | 12 (66.7%) | 12 (66.6%) | 10 (55.6%) | 8 (44.4%) | 9 (50.0%) | 7 (38.9%) | 0 (0%) | | |

+ or −: presence or absence of instability in microsatellite marker; MSI+ or MSI−: microsatellite instability positive or negative tumors, respectively

[a] Microsatellite markers marked in *bold* represent NCI reference panel

**Table 2** Frequency of loss of heterozygosity (LOH) in endometrial carcinomas

| Tumors No. | Microsatellite markers[a] | | | | | | | | | MSI |
|---|---|---|---|---|---|---|---|---|---|---|
| | **D2S123** | D3S966 | D17S855 | **BAT-25** | D17S1323 | **D5S346** | **D17S250** | D18S58 | **BAT-26** | |
| 1 | LOH[b] | – | – | LOH | – | LOH | – | – | – | + |
| 2 | LOH | – | LOH[b] | – | – | – | – | – | – | + |
| 3 | – | LOH[b] | – | – | – | – | – | – | – | + |
| 4 | LOH[b] | LOH | – | LOH | – | – | – | – | – | + |
| 5 | – | – | LOH | – | LOH | – | – | – | – | + |
| 6 | – | – | – | – | – | LOH | – | – | – | + |
| 7 | LOH | – | – | – | – | – | – | – | – | + |
| 8 | – | – | LOH | – | – | – | – | – | – | – |
| 9 | LOH | – | – | – | – | – | – | – | – | – |
| 10 | – | – | LOH | – | LOH | – | – | – | – | – |
| 11 | LOH | – | – | – | – | – | – | – | – | – |
| 12 | – | LOH | – | – | – | – | – | – | – | – |
| 13 | – | LOH | – | – | – | – | – | – | – | – |
| 14 | – | – | – | – | LOH | – | – | – | – | – |
| 15 | – | – | – | LOH | – | – | – | – | – | – |
| No. of tumors with LOH | 6 | 4 | 4 | 3 | 3 | 2 | 0 | 0 | 0 | |
| % of total ($n = 56$) | 10.7% | 7.1% | 7.1% | 5.4% | 5.4% | 3.6% | 0% | 0% | 0% | |

+: presence of microsatellite instability (MSI); –: absence of LOH or MSI

[a] Microsatellite markers marked in *bold* represent NCI reference panel

[b] Presence of LOH and MSI simultaneously in the same microsatellite marker

**Table 3** *PTEN* gene mutations in endometrial carcinomas with (MSI+) and without (MSI–) microsatellite instability

| Tumors No. | MSI+ ($n = 18$) | | | | Tumors No. | MSI– ($n = 38$) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Exon | Codon/ nucleotide | Mutation | Predicted effect | | Exon | Codon/ nucleotide | Mutation | Predicted effect |
| 1 | 1 | 6/17-18 | Del AA | Stop 9 | 1 | 2 | 28/83 | Del T | Stop 53 |
| 2 | 3 | 67/187-88 | Del AA | Stop 72 | 2 | 4 | 73-4/219-22 | Del AAGA | Stop 98 |
| 3 | 3 | 56/167 | TTT → TGT | Phe56Cys | 3 | 3 | 67/187-88 | Del AA | Stop 72 |
| | 5 | 130/389 | CGA → CAA | Arg130Gly | | 5 | 155/464 | TAT → TGT | Tyr155Cys |
| 4 | 5 | 105/313 | TGT → CGT | Cys105Arg | 4 | 5 | 130/389 | Del G | Stop 133 |
| 5 | 5 | 130/388 | CGA → GGA | Arg130Gly | 5 | 5 | 130/388 | CGA → TGA | Arg130Stop |
| 6 | 5 | 130/388 | CGA → GGA | Arg130Gly | 6 | 5 | 130/389 | CGA → CAA | Arg130Gln |
| 7 | 5 | 164/491 | Del A | Stop 166 | 7 | 5 | 130/388 | CGA → GGA | Arg130Gly |
| 8 | 5 | 130/389 | CGA → CAA | Arg130Gln | 8 | 5 | 130/389 | CGA → CAA | Arg130Gln |
| | 7 | 233/697 | CGA → TGA | Arg233Stop | | 8 | 299/895 | GAA → TAA | Glu299Stop |
| 9 | 7 | 233/697 | CGA → TGA | Arg233Stop | 9 | 5 | 130/389 | Del G | Stop 133 |
| **10** | **8** | **321/963** | **Ins A** | **Stop 324** | 10 | 5 | 112/334 | CTA → GTA | Leu112Val |
| 11 | 8 | 288/922 | GAA → TAA | Gly288Stop | 11 | 5 | 130/388 | CGA → GGA | Arg130Gly |
| | 5 | 130/388 | CGA → GGA | Arg130Gly | 12 | 5 | 130/388 | CGA → TGA | Arg130Stop |
| 12 | 8 | 328–334 | Del 20 bp | Stop 342 | 13 | 5 | 130/388 | CGA → GGA | Arg130Gly |
| | 7 | 241/721–22 | Del TT | Stop 242 | | | | | |
| 13 | 8 | 319/956 | Ins A | Stop 324 | 14 | 7 | 233/697 | CGA → TGA | Arg233Stop |
| 14 | 8 | 317-18/950-3 | Del TACT | Stop 320 | **15** | **8** | **321/963** | **Del A** | **Stop 343** |

*Bold data*, tumors with mutation in mononucleotide poly(A)$_6$ microsatellite sequence

three cases of MSI– tumors. Two MSI+ and two MSI– tumors had a change of G389A in codon 130, leading to the substitution of Arg130 with Gln. In three other cases (twice in MSI+ and once in MSI–), C697T change repeated in codon 233 (exon 7), causing premature stop codon. In one MSI+ carcinoma and one MSI– tumor, the same deletion of 187-188AA occurred in 56 codon (exon 3). A detailed description of all mutations in the *PTEN* gene is presented in Table 3.

Mutations in the *PTEN* gene occurred significantly more frequently in MSI+ (14/18, 77.8%) tumors than in MSI– (15/38, 39.5%) tumors ($p = 0.007$). However, only in one MSI+ carcinoma, mutation was identified in the region of the *PTEN* gene, characteristic for microsatellite instability, i.e., in repeat sequence

poly(A)$_6$ located in exon 8 in 321–323 codons and mutation in the same sequence was also identified in one MSI− tumor (bold data, Table 3).

## Mutations in beta-catenin (*CTNNB1*) gene

In Table 4, mutations identified in exon 3 of the *CTNNB1* gene are presented. They were found in 9 of 56 (16.1%) endometrial carcinomas. All mutations were single-nucleotide missense mutations causing amino acid change in beta-catenin protein. Among the nine mutations, eight were located within the GSK-3beta consensus site, affecting serine and threonine residues at codons: 33, 37 and 41. In four tumors, Ser37 was changed to Cys or Phe or Tyr by the alteration of TCT at codon 37 to TGT, TTT or TAT, respectively. Three other cases had change of ACC at codon 41 to ATC or GCC, altering Thr41, respectively, to Ile or Ala. In one case, mutation of TCT/TGT at codon 33 changed Ser33 to Cys. Besides that, one point mutation (GGA/GAA) occurred at codon 34, flanking the Ser in codon 33, as a consequence of which Gly34 was changed to Glu.

The incidences of *CTNNB1* gene mutations did not differ significantly between themselves in MSI+ (3/18, 16.7%) and MSI− (6/38, 15.8%) tumors ($p = 0.760$). In seven of nine (77.8%) carcinomas, in which mutations were identified in the *CTNNB1* gene, mutations in the *PTEN* gene occurred simultaneously (Table 4).

## MSI, *PTEN*, *CTNNB1* mutations: correlations with clinico-pathological features of tumors

The frequency of the occurrence of mutations in the MSI, *PTEN* and *CTNNB1* genes were compared with the selected clinical and histopathological features of the examined tumors. These results have been presented in Table 5. The evaluated cases of endometrial carcinomas were obtained from patients between 29 and 80 years of age. MSI as well as mutations in the *PTEN* or *CTNNB1* genes were more common among patients below 60 years of age as compared to older

**Table 4** Mutations in exon 3 of *CTNNB1* gene in endometrial carcinomas

| Tumor | Codon | Base change | Predicted effect | Coexistence of mutation in the *PTEN* gene |
|---|---|---|---|---|
| 1 | 33 | TCT → TGT | Ser 33 Cys | No mutation |
| 2 | 34 | GGA → GAA | Gly 34 Glu | Exon 5/missense<br>Exon 8/nonsense |
| 3 | 37 | TCT → TGT | Ser 37 Cys | Exon 7/del 2 bp<br>Exon 8/del 20 bp |
| 4 | 37 | TCT → TTT | Ser 37 Phe | Exon 3/del 2 bp<br>Exon 5/missense |
| 5 | 37 | TCT → TAT | Ser 37 Tyr | Exon 1/del 2 bp |
| 6 | 37 | TCT → TTT | Ser 37 Phe | Exon 8/del 1 bp |
| 7 | 41 | ACC → ATC | Thr 41 lle | Exon 4/del 4 bp |
| 8 | 41 | ACC → ATC | Thr 41 lle | No mutation |
| 9 | 41 | ACC → GCC | Thr 41 Ala | Exon 8/ins 1 bp |

**Table 5** Correlations between microsatellite instability (MSI+) and *PTEN* and *CTNNB1* gene mutations and clinico-pathological features of endometrioid endometrial carcinomas

| Clinico-pathological data | No. of tumors | No. of MSI+ (%) | $p$[a] | No. of *PTEN* mutations (%) | $p$[a] | No. of *CTNNB1* mutations (%) | $p$[a] |
|---|---|---|---|---|---|---|---|
| Total | 56 | 8 (32.1) | | 29 (51.8) | | 9 (16.1) | |
| Patients age[b] (years) | | | | | | | |
| ≤60.5 | 24 | 11 (45.8) | 0.058 | 16 (66.7) | 0.054 | 5 (20.8) | 0.536 |
| >60.5 | 32 | 7 (21.8) | | 13 (40.0) | | 4 (11.4) | |
| FIGO stage | | | | | | | |
| I | 41 | 14 (34.1) | I vs. II–IV | 23 (56.1) | I vs. II–IV | 8 (19.5) | I vs. II–IV |
| II–IV | 15 | 4 (26.7) | 0.836 | 6 (40.0) | 0.286 | 1 (5.6) | 0.454 |
| Histological grade | | | | | | | |
| G1 | 19 | 3 (15.8) | G3 vs. G1 + G2 | 10 (52.6) | G3 vs. G1 + G2 | 4 (21.1) | G3 vs. G1 + G2 |
| G2 | 28 | 9 (32.1) | 0.042 | 11 (39.2) | 0.039 | 4 (13.8) | 0.958 |
| G3 | 9 | 6 (66.7) | | 8 (88.9) | | 1 (9.1) | |

[a] $\chi^2$ -test (Yates' correction)

[b] The median age was used as cut-off point

patients (45.8 vs. 21.8%; 66.7 vs. 40.0%, 20.8 vs. 11.4%, respectively), but differences were not statistically significant.

MSI and mutations in the *PTEN* or *CTNNB1* genes tended to be more frequent in the tumors in FIGO stage I as compared to more advanced tumors (FIGO stage I vs. stages II–IV: 34.1 vs. 26.7%; 56.1 vs. 40.0%; 19.5 vs. 5.6%, respectively), although this was not statistically significant. MSI and *PTEN* gene mutations were, however, significantly more common in the tumors with the lowest morphological differentiation than in well and moderately differentiated cases (G3 vs. G1 + G2: $p = 0.042$; $p = 0.039$; respectively). The incidence of *CTNNB1* gene mutations demonstrates no dependence on the histological grade of tumors.

## Discussion

We demonstrated in this study that microsatellite instability occurred in 18 out of 56 endometrioid carcinomas of endometrium (MSI+ 32.1%), which suggests that it is a significant molecular event associated with the pathogenesis of about one-third of these tumors. Our results are consistent with those reported by other authors in which the frequency of MSI in sporadic endometrial carcinomas generally was from 20 to 35% of cases (Tibiletti et al. 1999; Fiumicino et al. 2001; McDonald et al. 2000; Muresu et al. 2002). In the studied microsatellite loci, the loss of heterozygosity (LOH) was also identified in 26.8% of carcinomas. The occurrence of microsatellite instability in a tumor together with loss of heterozygosity could be an additional evidence of increasing DNA instability and carcinogenesis progression.

Microsatellite instability positive phenotype is a common feature of some tumor types, such as hereditary colon and gastric carcinomas arising in HNPCC syndrome, as well as their sporadic forms (Boland et al. 1998; Lawes et al. 2003). Microsatellite instability is a consequence of the loss of the DNA repair mechanism due to mutations in mismatch repair genes and is associated with accumulation of frameshift mutations in repetitive sequences of some particular target oncogenes or tumor suppressor genes, which can cause functional alterations in genome leading to carcinogenesis. Although the frequency of MSI in sporadic endometrial carcinomas is relatively high, the molecular genetic targets for MSI development in these neoplasms are poorly known.

In our series of endometrial carcinomas, MSI and/or LOH occurred in dinucleotide repeats, most frequently located in regions of *MLH1*, *MSH2* or *BRCA1* and less frequently in regions of *DCC* and *APC* genes and in mononucleotide repeat located in region of *KIT* gene. On the other hand, we demonstrated the absence of mutations in BAT26 mononucleotide microsatellite sequence, and in our other studies no mutations were found in the coding $(A)_{10}$ repeated sequence of *TGF-βRII* gene (personal data, non-published). However, it is known from literature data that both these sequences are almost always mutated in MSI+ colon and gastric tumors (Markowitz et al. 2000; Baldinu et al. 2002). It follows that in sporadic endometrial carcinomas, other genes may be mutational targets for MSI.

Mutations in the *PTEN* and *CTNNB1* (encoding beta-catenin) genes are molecular manifestations of sporadic endometrial carcinomas (Risinger et al. 1998; Machin et al 2002). Therefore, we considered it interesting to investigate the association between occurrence in the studied cases of mutations in those genes and microsatellite instability. We demonstrated that 51.8% of the tumors had mutations in the *PTEN* gene and only 16.1% had mutations in the beta-catenin gene, which is comparable with results reported by others authors (Risinger et al. 1998; Machin et al. 2002; Salvesen et al. 2004). The occurrence of mutations in the *PTEN* gene was evidently related with the occurrence of microsatellite instability. We demonstrated that in MSI+ tumors, mutations in the *PTEN* gene were significantly more frequent than in the MSI− tumors (77.8 and 39.5%, respectively; $p = 0.007$). On the other hand, we found no such relation between the incidence of MSI and mutations in the beta-catenin gene (16.7 and 15.8%; $p = 0.760$).

The oncogenic activity of beta-catenin, the key protein of Wnt signal pathway, encoded by the *CTNNB1* gene, is associated with many tumors (Bullions and Levine 1998; Mirabelli-Primdahl et al. 1999; Stambolic 2002), but its role in the pathogenesis of sporadic endometrial carcinomas is not fully understood. In our series of endometrioid endometrial carcinomas, mutations in the *CTNNB1* gene were detected most frequently in FIGO stages I and II and histological grades 1 and 2, indicating an early event in carcinogenesis. All but one *CTNNB1* mutations found in our tumors occurred in the sequences encoding serine 33 and 37, or threonine 41, that are involved in GSK-3β-dependent phosphorylation of beta-catenin. The biological effects of those alterations include accumulation of free beta-catenin, leading to pathological proliferation of cells. Therefore, localization of *CTNNB1* mutations in serine/threonine residues in our tumors may additionally suggest their important role in endometrial tumorigenesis.

Regarding the relation between mutations in the *CTNNB1* gene and MSI, the opinion of most researchers is concordant, that in sporadic endometrial carcinomas there is no significant association between these two genetic defects (Mirabelli-Primdahl et al. 1999; Machin et al. 2002). Our results are similar, indicating that beta-catenin and MSI can follow two different pathways in endometrial carcinogenesis. Interestingly, mutations in the *CTNNB1* gene, detected in our study, most frequently (7/9; 77.8%) coexisted in the same tumor with mutations in the *PTEN* gene. Recently, Persad et al. (2001) demonstrated that *PTEN* had the ability to regulate localization of free beta-catenin in prostate carcinoma cell lines, suggesting that these two events within the same pathway may cooperate with each other in prostate carcinogenesis. To date only Wappenschmidt et al. (2004) have studied the relations of *PTEN* and beta-catenin abnormalities in endometrial carcinoma and they failed to find similar relations in this neoplasm. In our series, we detected a subgroup of endometrioid endometrial carcinomas (7 of 56, 12.5%) that showed mutations in both the *PTEN* and beta-catenin genes. This might indicate that both molecular alterations could be involved in the development of a subset of these carcinomas through the same molecular pathway. However, it must be emphasized that our number of tumors with mutation in the *CTNNB1* gene was small. Thus, further studies on larger group of tumors with additional analysis of beta-catenin expression are needed to verify this observation.

Mutational inactivation of the *PTEN* tumor suppressor gene is the most frequent genetic defect identified as yet in sporadic endometrial carcinomas, since it occurs in these tumors from 34 to 83% depending on sample selection (Risinger et al. 1998; Mutter et al. 2000; Salvesen et al. 2004).

The frequency and distribution of *PTEN* gene mutations in our present and previous series of endometrial carcinomas are consistent with those of others, because they are within 48.5–51.8% range (Konopka et al. 2002, 2004). *PTEN* mutations occur at higher rates in MSI+ tumors (Kong et al. 1997; Bussaglia et al. 2000; Baldinu et al. 2002), but the biological significance of this association remains unclear. The coding region of *PTEN* contains two poly$(A)_6$ microsatellite tracts in exons 7 and 8. Recently, mutations at these repeat sequences of *PTEN* were reported in colorectal tumors showing microsatellite instability, suggesting that *PTEN* might be a mutational target for MSI in carcinomas with defective mismatch repair function (Shin et al. 2001). However, in line with Gurin et al. (1999) and other authors (Cohn et al. 2000), the presence of mutations in the *PTEN* gene poly$(A)_6$ tracts in our study was very rare. Among 18 MSI+ endometrial carcinomas, 14 had mutations in the *PTEN* gene, but only one of them occurred in the microsatellite locus $(A)_6$ in exon 8. The same sequence was also mutated in one MSI− tumor. The remaining *PTEN* mutations in MSI+ and MSI− cases had a similar spectrum and were not attributable to MSI. Besides that, mutations in the *PTEN* gene were almost twice more frequent than MSI (in 29 and 18 cases, respectively). In addition, they occurred in 39.5% of endometrial carcinomas with stable DNA. The detection of the *PTEN* gene mutations in MSI negative endometrial carcinomas indicates that *PTEN* defect may precede the development of MSI in these tumors, independently of mismatch repair deficiency. Recent studies by Wang et al. (2002) on mouse $PTEN^{+/-}/Mlh1^{-/-}$ model demonstrated that the mutation of one allele of the *PTEN* gene was sufficient for the initiation of endometrial carcinogenesis, while a defect of the *MLH1* mismatch repair gene was responsible for the loss of the wild allele of the *PTEN* gene, which resulted in acceleration of the malignant process. The significant association of *PTEN* mutations with MSI suggests that these two defects are involved in carcinogenesis of subset of endometrioid endometrial tumors through the same molecular pathway in which MSI may be an additional event to *PTEN* inactivation, probably resulting in increasing of tumor aggressiveness due to accumulation of errors. Most authors agree that *PTEN* mutations in endometrial carcinomas are mainly associated with endometrioid subtype of tumors in FIGO stages I and II and histological grades 1 and 2 (Risinger et al. 1998; Bussaglia et al. 2000; Salvesen et al. 2004). Microsatellite instability, however, has been observed equally frequently in both early and advanced (FIGO stage III, G3) edometrioid endometrial carcinomas (Tibiletti et al. 1999; Fiumicino et al. 2001; Muresu et al. 2002) and also in accompanied lymph node metastasis (Ohwada et al. 2002). In our series of endometrioid carcinomas, mutations in *PTEN* gene and MSI were found in all FIGO stages, although they tended to be more common in tumors with the lowest stage. On the other hand, this study showed that *PTEN* mutations as well as MSI were significantly more frequent in poorly differentiated than in moderately and well-differentiated tumors, suggesting that these defects may be associated with a loss of ability of endometrial cells for differentiation and therefore may play some role in increasing its malignancy.

In conclusion, the results of this study indicate that the most frequently occurring mutations in the *PTEN* gene may be a key event for tumorigenesis in a significant fraction of endometrioid endometrial carcinomas.

The coexistence with them or absence of mutations in the *CTNNB1* gene or MSI suggest that carcinogenesis of these tumors can, then, follow alternative pathways. One of them is related to genetic instability, the other pathway goes without MSI but with the participation of mutations in the *CTNNB1* gene. These events may provide new insights into the biology of endometrial carcinoma, which still requires further research.

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