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Microsatellite Instability (MSI) Analysis in Patients with Endometrial Cancer*

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Microsatellite instability (MSI) seems to be important for the development of various human cancers including sporadic endometrial cancer. The aim of this study was evaluation of microsatellite instability in 20 postmenopausal women with endometrial adenocarcinoma in DNA samples obtained from cancer tissue and blood of the same patients. Control DNA was obtained from normal endometrial tissue (n=25). MSI was studied at five loci containing single- or dinucleotide repeat sequences and mapping to different chromosomal locations: BAT-25 (at locus 4q12), BAT-26 (2p16), D2S123 (2p16-p21), D5S346 (5q21-q22) and D17S250 (17q11.2-q12). No differences in the MSI frequencies between blood and cancer tissue obtained from patients were detected. The microsatellite instability status was significantly higher in endometrial cancer tissue [5/20 (25%)] as compared to control [3/25 (12%)] ($p<0.05$). There were no significant differences between MSI presence in the subgroups assigned to the histological grades ($p>0.05$). The results suggest that the microsatellite instability seems to be important in the development of sporadic endometrial cancer.

Introduction

Microsatellite sequences are short repeat nucleotide sequences disseminated in whole genome in normal conditions. In *Eucaryota* genome there are repeated sequences consisting of one, two, three and four nucleotides. Over 90% of the till this moment studied microsatellite sequences from mononucleotides to tetranucleotides show polymorphism. Small deletions or expansions in tumor DNA, manifested as shifts in allelic electrophoresis mobility characterize microsatellite instability (MSI). Genetic instability is considered to be responsible for a rapid accumulation of somatic mutations in various tumor suppressor genes and oncogenes, thus playing an important role in the initiation and progression of malignant tumors [16, 17].

Previous studies have indicated that MSI seems to be important in the development of various human cancers [2, 25, 33] including sporadic endometrial cancer (EC) [7, 9, 21, 31].

Endometrial cancer is one of the most common malignant neoplasms, which appear in uterine body [26]. About 80% of the cases are diagnosed after menopause. The highest incidence estimated in 57–58 years is moving to 6 and 7 decade of life at present [4]. Endometrial cancer is fourth the most common female carcinoma [29]. Annually 150,000 new cases of this cancer are noted worldwide. Every year 65 new cases of endometrial cancer in age group 65–75 years are diagnosed among every 100,000 women.

Some risk factors have been identified, related to reproduction (such as early age at menarche, late age at menopause and nulliparity) or more immediately estrogen-related (i.e. conditions such as the polycystic ovarian syndrome) [4, 30].

Prognostic factors important in the evaluation of EC are: tumor size, tumor grade, presence or absence and depth of myometrial invasion, myometrial lymphatic/vascular space invasion, steroid hormone receptor status, ploidy and proliferative indices. However, these factors present an incomplete picture of the tumor biology. Therefore, investigation of other prognostic factors is of special clinical relevance, particularly in view of the unexpectedly progressive course of the disease and frequent relapses in some cases.

MSI is characterized by small insertions or deletions within short tandem repeats in tumor DNA when compared to the corresponding normal DNA. MSI was firstly demonstrated in patient with hereditary nonpolyposis colorectal carcinoma (HNPCC), an inherited cancer syndrome that

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predisposes also to endometrial cancer, which represents the second most common malignancy in HNPCC families [14, 24]. Later studies have also demonstrated that MSI is present in about 20% of sporadic colorectal tumors [20]. Endometrial cancer is one of the most common extracolonic tumors associated with HNPCC, and MSI has been reported to be present in 9–45% of sporadic cases [5, 6, 12, 19, 27].

In the present study MSI status in women with sporadic endometrial cancer was investigated.

Material and Methods

Endometrial cancer samples

Twenty patients with histologically-proven diagnosis of endometrial adenocarcinoma were included in the study (mean age ±SD – 63.75±4.72 years). Endometrial cancer was classified as sporadic after evaluation of patient's family history in order to exclude the presence of the HNPCC syndrome. Family history for cancer was evaluated by questionnaire interviews in endometrial cancer patients attending the Department of Obstetrics and Gynecology of the Medical University in Łódź after the initial surgical treatment. Tumor tissues and blood were obtained from postmenopausal women with endometrial adenocarcinoma treated at Department of Obstetrics and Gynecology between 2002 and 2004. Tissues were frozen immediately and stored at -70°C. All tumors were staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). There were 7 tumors of I stage, 6 of II stage and 7 of III stage in total. DNA from normal endometrial tissue (n=25) served as a control.

DNA isolation

DNA was extracted from fresh material using commercially available QIAmp Kit (Qiagen GmbH, Hilden, Germany) DNA purification kit according to manufacturer's instruction.

Microsatellite analysis

Tumor DNA and corresponding normal DNA were analyzed using a panel of five microsatellite markers for mononucleotide and dinucleotide repeat sequences: BAT25 (at locus 4q12), BAT26 (2p16), D2S123 (2p16-p21), D5S346 (5q21-q22) and D17S250 (17q11.2-q12) [3]. All primer sequences were as reported in Genome DataBase (GDB, at: http://www.gdb.org).

The PCR was carried out in a Perkin-Elmer/Gene Amp, PCR System 2400 thermal cycler. The thermal cycling conditions were 60s at 94°C, 60s at 60°C, 60s at 72°C, repeated for 30 step cycles. PCR amplification was performed in a final volume of 25 µl. The reaction mixture contained 5 ng of genomic DNA, 0.2 µmol of each appropriate primer (ARK Scientific GmbH Biosystems, Darmstad, Germany), 2.5 mM $MgCl_2$, 1 mM dNTPs and 1 unit of Taq Polymerase (Qiagen GmbH, Hilden, Germany). PCR products were fractionated by denaturing electrophoresis in a 6% polyacrylamide gel (PAGE) and visualized by silver staining.

Statistical analysis

For statistical analysis, the χ^2 test was performed, $p<0.05$ was considered significant.

Results

A sample was classified as MSI-high (MSI-H) if two or more markers showed instability, MS-stable (MSS) if no instability was noted, and MSI-low (MSI-L) if a single marker revealed novel bands compared with the corresponding normal DNA.

TABLE 1

Number of endometrial cancer patients and control with the presence or absence of microsatellite instability (MSI)

MSI status	Endometrial cancer patients (n=20)		Control (n=25)	
	Number	Frequency	Number	Frequency
MSI+	**5**	**0.25***	**3**	**0.12***
MSI-H	4	0.20	1	0.04
MSI-L	1	0.05	2	0.08
MSS	**15**	**0.75**	**22**	**0.88**

*$p<0.05$ as compared to controls

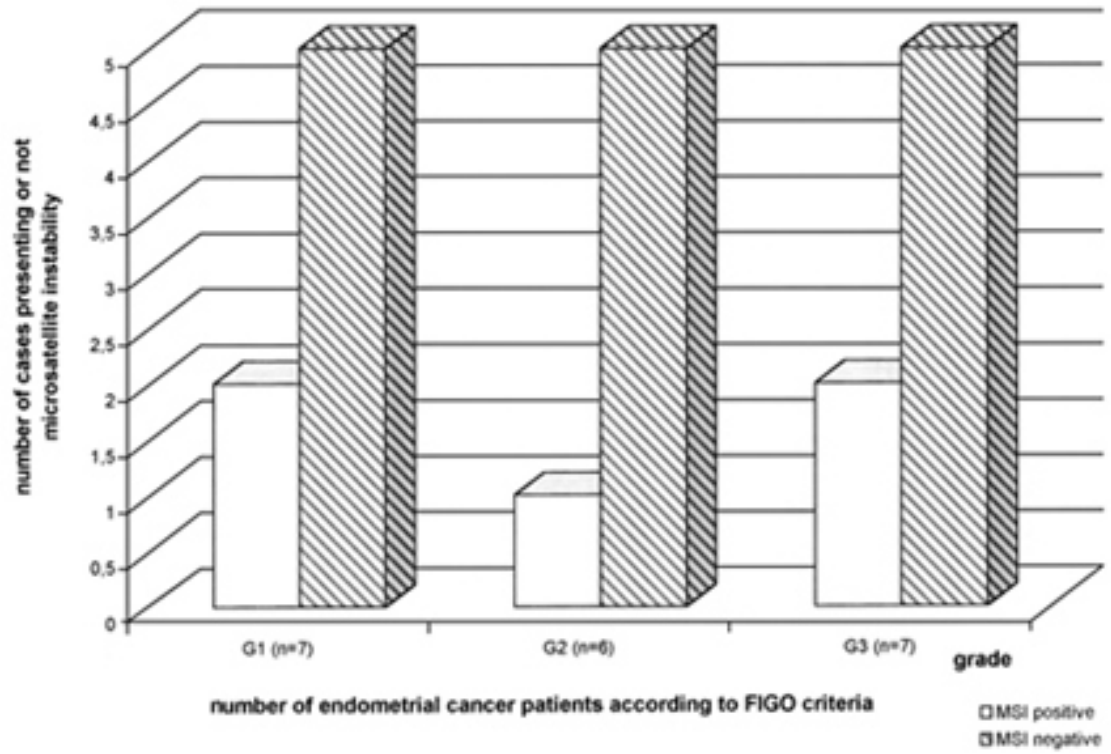

Fig. 1. Number of endometrial cancer patients (n=20) presenting or not microsatellite instability in relation to tumor grade.

MSI was determined in 20 endometrial carcinoma tissues and blood from patients and in 25 control samples. There were no differences in MSI presence between blood and cancer tissue obtained from the same endometrial cancer patients.

Five out of 20 (25%) tumors tested were found to be MSI positive, 4 MSI-high and 1 MSI-low (Table 1). It can be seen from the Table 1 that there were significant differences in MSI frequency between women with endometrial cancer and control. The frequency of MSI presence in cancer samples was higher than in normal samples ($p<0.05$).

A dependency of the MSI frequency distribution on the tumor grade evaluated according to FIGO criteria in women with endometrial cancer is displayed in Figure 1. The histological analysis of tumor grade showed a lack of association ($p>0.05$) between tumor grade and microsatellite instability presence.

Discussion

Endometrial tumorigenesis is still poorly understood. The development of endometrial cancer is associated with an accumulation of specific genetic alterations [22]. The activation of oncogenes, the loss or inactivation of repressor genes and impaired mismatch-repair function are known to be involved in the development of tumors. It is well known, that MSI plays an important role in the pathogenesis and disease progression of endometrial cancer [1, 11, 15, 23, 18].

Many studies have documented that MSI may be a marker of a tendency for replication errors in human cancers [13, 23, 31]. Defects in DNA mismatch-repair genes such as MLH1, MSH2 and MSH6 lead to replication errors revealed as instability in microsatellite markers. Several studies have indicated that loss of MLH1 expression can account for a large proportion of sporadic endometrial tumors [8, 10, 28]. In the majority of endometrial tumors with high MSI the loss of MLH1 expression was found, whereas loss of expression was less frequent in tumors with intermediate MSI. Thus, pathological expression of MLH1 does not seem to account for all tumors with a MSI-positive phenotype, indicating that other mismatch repair genes might also be involved [28].

In the light of substantial evidence that the progression of endometrial cancer can be associated with microsatellite instability, it seems reasonable to check a possible correlation between MSI and clinical status of endometrial cancer patients. The patients included in the study received no chemotherapy or hormone therapy. In this work conducted on 20 endometrial carcinoma patients we find a correlation between MSI and occurrence of cancer. Twenty-five per cent of endometrial cancer patients presented MSI, while only 3 normal samples from 25 healthy individuals (12%) showed this pattern.

However, the histological analysis of tumor grade showed a lack of correlation between tumor grade and the number of patients presenting MSI.

Recent studies have suggested that genetic alterations, including *p53* mutations, loss of heterozygosity, and *K-ras*, *PTEN* and *Her2/neu* gene amplification, can be detected in endometrial cancer [17]. Our findings provide additional evidence that genetic alterations, including MSI, may occur as relatively early events in the development of endometrial cancer.

The implications of MSI in DNA from patients with endometrial cancer may be associated with different tumorigenic pathways. The genome-wide MSI may be correlated to the existence of pathogenetic mechanisms inducing progressive accumulation of sequence errors and providing a selective advantage during malignant evolution.

Our study implies that it is possible that the MSI process may be involved in the appearance and/or progression of endometrial cancer. Further studies, conducted on a larger population, are required to clarify this point.

References

1. *Arzimanoglou II, Gilbert F, Barber HRK:* Microsatellite instability in human solid tumors. Cancer 1998, 82, 1808-1820.
2. *Benachenhou N, Guiral S, Gorska-Flipot I, Labuda D, Sinnett D:* Frequent loss of heterozygosity at the DNA mismatch-repair loci hMLH1 and hMSH3 in sporadic breast cancer. Br J Cancer 1999, 79, 1012-1017.
3. *Boland CR, Thibodeau SN, Hamilton SR:* National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. Cancer Res 1998, 58, 5248-5257.
4. *Bremond A, Bataillard A, Thomas L:* Cancer of the endometrium. French National Federation of Cancer (FNCLCC). Br J Cancer 2001, 84, 31-36.
5. *Caduff RF, Johnston CM, Svoboda-Newman SM, Poy EL, Merajver SD, Frank TS:* Clinical and pathological significance of microsatellite instability in sporadic endometrial carcinoma. Am J Pathol 1996, 148, 1671-1678.
6. *Catasus L, Machin P, Matias-Guiu X, Prat J:* Microsatellite instability in endometrial carcinomas: clinicopathologic correlations in a series of 42 cases. Hum Pathol 1998, 29, 1160-1164.
7. *Chadwick RB, Pyatt RE, Niemann TH, Richards SK, Johnson ChK, Stevens MW, Meek JE, Hampel H, Prior TW, Chapelle A:* Hereditary and somatic DNA mismatch repair gene mutations in sporadic endometrial carcinoma. J Med Genet 2001, 38, 461-466.
8. *Esteller M, Levine R, Baylin SB, Ellenson LH, Herman JG:* MLH1 promoter hypermethylation is associated with the microsatellite instability phenotype in sporadic endometrial carcinomas. Oncogene 1998, 17, 2413-2417.
9. *Furlan D, Casati B, Cerutti R, Facco C, Terracciano L, Capella C, Chiaravalli AM:* Genetic progression in sporadic endometrial and gastrointestinal cancers with high microsatellite instability. J Pathol 2002, 197, 603-609.
10. *Gurin CC, Federici MG, Kang L, Boyd J:* Causes and consequences of microsatellite instability in endometrial carcinoma. Cancer Res 1999, 59, 462-466.
11. *Hardisson D, Moreno-Bueno G, Sanchez L, Sarrio D, Suarez A, Calero F, Palacios J:* Tissue microarray immunohistochemical expression analysis of mismatch repair (hMLH1 and hMSH2 genes) in endometrial carcinoma and atypical endometrial hyperplasia: relationship with microsatellite instability. Mod Pathol 2003, 16, 1148-1158.
12. *Helland A, Borresen-Dale AL, Peltomaki P, Hektoen M, Kristensen GB, Nesland JM, de la Chapelle A, Lothe RA:* Microsatellite instability in cervical and endometrial carcinomas. Int J Cancer 1997, 70, 499-501.
13. *Ilyas M, Straub J, Tomlinson IP, Bodmer WF:* Genetic pathways in colorectal and other cancers. Eur J Cancer 1999, 35, 1986-2002.
14. *Ionov Y, Peinado MA, Malkhosyan S, Shibata D, Perucho M:* Ubiquitous somatic mutations in simple repeated sequences reveal a new mechanism for colonic carcinogenesis. Nature 1993, 363, 558-561.
15. *Katabuchi H, van Rees B, Lambers AR, Ronnett BM, Blazes MS, Leach FS, Cho KR, Hedrick P:* Mutations in DNA mismatch repair genes are not responsible for microsatellite instability in most sporadic endometrial carcinomas. Cancer Res 1995, 55, 5556-5560.
16. *Kinzler KW, Vogelstein B:* Lessons from hereditary colorectal cancer. Cell 1996, 87, 159-170.
17. *Lax SF:* Molecular genetic pathways in various types of endometrial carcinoma: from a phenotypical to a molecular-based classification. Virchows Arch 2004, 4, 213-223.
18. *Liu VW, Yang HJ, Wang Y, Tsang PC, Cheung AN, Chiu PM, Ng TY, Wong LC, Nagley P, Ngan HY:* High frequency of mitochondrial genome instability in human endometrial carcinomas. Br J Cancer 2003, 89, 697-701.
19. *MacDonald ND, Salvesen HB, Ryan A, Iversen OE, Akslen LA, Jacobs IJ:* Frequency and prognostic impact of microsatellite instability in a large population-based study of endometrial carcinomas. Cancer Res 2000, 60, 1750-1752.
20. *Marra G, Boland CR:* Hereditary nonpolyposis colorectal cancer: the syndrome, the genes, and historical perspectives. J Natl Cancer Inst 1995, 87, 1114-1125.
21. *Muresu R, Sini MC, Cossu A, Tore S, Baldinu P, Manca A:* Chromosomal abnormalities and microsatellite instability in sporadic endometrial cancer. Eur J Cancer 2002, 38, 1802-1809.
22. *Mutter GL:* Diagnosis of premalignant endometrial disease. J Clin Pathol 2002, 55, 326-331.
23. *Parc YR, Halling KC, Burgart LJ, Mc Donnel SK, Schaid DJ, Thibodeau SN*: Microsatellite instability and hMLH1/hMSH2 expression in young endometrial carcinoma patients: associations with family history and histopathology. Int J Cancer 2000, 86, 60-66.
24. *Peltomaki P, Lothe RA, Aaltonen LA:* Microsatellite instability is associated with tumors that characterize the hereditary non-polyposis colorectal carcinoma syndrome. Cancer Res 1993, 53, 5853-5855.
25. *Pinto M, Oliveira C, Machado JC, Cirnes L, Carneiro F, Hamelin R:* MSI-L gastric carcinomas share the hMLH1 methylation status of MSI-H carcinomas but not their clinicopathological profile. Lab Invest 2000, 80, 1915-1923.
26. *Rose PG:* Endometrial carcinoma. N Engl J Med 1996, 335, 610-619.
27. *Sakamoto T, Murase T, Urushibata H, Kato K, Takada H, Imamura T, Mori H, Wake N:* Microsatellite instability and somatic mutations in endometrial carcinomas. Gynaecol Oncol 1998, 71, 53-58.
28. *Salvesen HB, MacDonald N, Ryan A, Iversen OE, Jacobs IJ, Akslen LA, Das S:* Methylation of hMLH1 in a population-based series of endometrial carcinoma. Clin Cancer Res 2000, 6, 3607-3613.
29. *Simpkins SB, Bocker T, Swisher EM, Mutch DG, Gersell DJ, Kovatich HJ, Palazzo JP, Fishel R, Goodfellow PJ:* MLH1 promoter methyla-

tion and gene silencing is the primary cause of microsatellite instability in sporadic endometrial cancers. Hum Mol Genet 1999, 8, 661-666.
30. *Spaczyński M:* Onkologia Ginekologiczna. Urban &Partner, Wrocław 1997.
31. *Stefansson I, Akslen LA, MacDonald N, Ryan A, Das S, Jacobs IJ, Salvesen HB:* Loss of hMSH2 and hMSH6 expression is frequent in sporadic endometrial carcinomas with microsatellite instability: a population-based study. Clin Cancer Res 2002, 8, 138-143.
32. *Sturzeneker R, Bevilacqua RA, Haddad LA, Simpson AJ, Pena SD:* Microsatellite instability in tumors as a model to study the process of microsatellite mutations. Hum Mol Genet 2000, 9, 347-352.
33. *Thibodeau SN, Bren G, Schaid D:* Microsatellite instability in cancer of the proximal colon. Science 1993, 260, 816-819.

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