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Chromosomal abnormalities and microsatellite instability in sporadic endometrial cancer

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Abstract

Defective DNA mismatch repair and nonfunctional mechanisms controlling the proper progression of the cell cycle have been proposed as being responsible for the genomic instability and accumulation of karyotypic alterations in endometrial cancer (EC). To assess whether numerical chromosomal anomalies (aneuploidy) and microsatellite instability (MSI) might be representative of distinctive tumour behaviour, paraffin-embedded tissue samples from 86 patients with sporadic EC were evaluated by both fluorescence *in situ* hybridisation (FISH) and microsatellite analysis, using free nuclei and genomic DNAs (respectively). Approximately one-third of the tumours analysed (24/74; 32%) exhibited MSI, whereas 38/86 (44%) of the EC samples displayed aneuploidy. The majority of the unstable cases (15/24; 63%) were from advanced-stage patients. Conversely, 23 (61%) out of the 38 tumours with aneuploidy were from early-stage patients. No apparent correlation was found between MSI and aneuploidy, whereas the immunohistochemical (IHC) analysis revealed that inactivation of the *MLH1* mismatch repair gene may be involved in the majority of the MSI+ sporadic ECs. No genetic or cytogenetic alteration analysed here seems to add any significant predictive value to the stage of disease.

Keywords: Endometrial tumorigenesis; Fluorescence *in situ* hybridisation (FISH); Polymerase chain reaction (PCR); Microsatellite analysis; MLH1; MSH2; Prognosis

1. Introduction

Endometrial cancer (EC) is the leading cause of malignancy of the female genital tract, mostly affecting women in the post-menopausal age group [1]. EC rates vary worldwide and are highest in white women in Western populations [2]. Some risk factors have been identified, related to reproduction (such as early age at menarche, late age at menopause and nulliparity) or more directly oestrogen-related (i.e. conditions such as the polycystic ovarian syndrome) [2].

Endometrial tumorigenesis is still poorly understood. A sequential accumulation of genetic alterations from benign to malignant primary lesions has been hypothesised, following the model demonstrated for colorectal carcinoma [3,4]. For EC, several studies have implicated mutational inactivation of tumour suppressor genes, as well as activation of oncogenes, in its pathogenesis. In particular, disease-causing mutation of the *PTEN* [5,6] and *TP53* [7] tumour suppressor genes have been found in 10–38% of endometrial cancers [7–9], whereas activating alterations of the *K-ras* oncogene and overexpression of the *erb-B2* oncogene have been described in 10–20% of invasive endometrial carcinomas [10–12]. However, new putative tumour suppressor genes are expected to be involved in EC pathogenesis.

Although loss of heterozygosity (suggestive for the presence of tumour suppressor genes) has been found at various rates within different genomic regions, the highest frequency of allelic deletions was reported for chromosome 10q [13,14]. In particular, the regions 10q23 (where PTEN is located) [3,4] and 10q25-q26 [15,16] have been strongly correlated to endometrial

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tumorigenesis, as our group has also previously demonstrated in Ref. [17].

The increasing number of evidences indicates that a defective replication fidelity may represent an important molecular mechanism strongly involved in the genesis of different human malignancies, including EC. Tumours with a non-functional DNA mismatch repair display a genetic instability as inferred by detection of ubiquitous somatic variation in length of microsatellite sequences [18]. Microsatellite instability (MSI) is characterised by small insertions or deletions within short tandem repeats in tumour DNA when compared with the corresponding normal DNA. MSI was first demonstrated in patients with hereditary nonpolyposis colorectal carcinoma (HNPCC), an inherited cancer syndrome that also predisposes to EC, which represents the second most common malignancy in HNPCC families [18,19]. Frequency of MSI has been reported at various rates, ranging from 15 to 43% in sporadic ECs [20–23].

Rearrangements or deletions of almost every chromosomal arm have been widely described in EC [13,24,25]. However, gains of chromosomes 1 and 10 represent the most common cytogenetic abnormality detected in such a disease [24,25], as also demonstrated in our previous study in Ref. [26]. Such numerical abnormalities can be considered as an expression of aneuploidy [25] and, though not unanimously confirmed, seem to be due to chromosomal instability. In fact, several observations suggest that chromosomal instability is a necessary prerequisite for developing intratumoral DNA ploidy heterogeneity [27].

Prognosis of this tumour, which has implications on patient management, is determined by evaluation of the stage of disease, architectural grade, nuclear grade, myometrial invasion, and peritoneal cytology. These parameters have inherent subjectivity and, therefore, the search for an objective reliable parameter to determine prognosis is required. To assess whether aneuploidy and MSI might be representative of distinctive tumour phenotypes with different clinicopathological behaviour, we performed both fluorescence *in situ* hybridisation (FISH) and microsatellite analysis on archival tissues from a subset of 86 Sardinian patients with sporadic EC (incidence of EC in Sardinia has been reported similar to that of other Western countries, with 18.7 new cases per year per 100 000 inhabitants [28]). Statistical correlations between these genetic features and histopathological or clinical parameters were thus inferred.

2. Materials and methods

2.1. Tissue samples and DNA extraction

86 patients with histologically-proven diagnosis of endometrial cancer were included in the study. Patients were classified according to the presence of tumours confined to corpus uteri (stage I) or extended beyond corpus uteri (stages II and III), following the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) guidelines [29].

Patients were unrelated and originated from Sardinia. EC was classified as sporadic after evaluation of patients' family history in order to exclude the presence of the HNPCC syndrome [30]. Family history for cancer was evaluated by questionnaire interviews in EC patients attending the Department of Obstetrics and Gynecology at the University of Sassari after the initial surgical treatment. No significant evidence of tumours in first- and second-degree relatives was observed in our series of EC patients. Disease status at the time of diagnosis was defined depending on clinical staging as assessed by medical history, physical examination and instrumental tests. Clinical follow-up was performed over a median period of 49 months (range 12–87 months). Patients were informed about the aims of this study.

Genomic DNA was isolated from tumours samples and corresponding normal tissues, as previously described in Ref. [17]. The percentage of neoplastic and normal cells in each tissue specimen was estimated by light microscopy. Tumour samples were estimated to contain at least 80% intact neoplastic cells.

2.2. FISH analysis

Preparation of free nuclei from paraffin-embedded EC tissues and FISH were performed as previously reported in Ref. [26]. Briefly, two 40-μm tissue sections for each case were deparaffinised in xylene, rehydrated in distilled water, and proteinase K-digested. Nuclei were sedimented by centrifugation, suspended in phosphate-buffered saline (PBS), and spotted in the middle of several 3-aminopropyltriethoxysilane-treated slides. After air-drying the slides, nuclei were fixed on the glass by using absolute methanol.

For *in situ* hybridisation, DNA probes specific for highly repetitive sequences from peri-centromeric regions of chromosomes 1 and 10 were used as previously reported in Ref. [26]. To assess the specificity of aneuploidy for these two chromosomes, repetitive peri-centromeric sequences of chromosome 18 were used as controls. Hybridisation was detected by fluorescein isothio-cyanate (FITC)-conjugated avidin (Vector Laboratories) and/or rhodamine-conjugated antidigoxigenin antibodies (Boehringer), with no signal amplification. Nuclei were counterstained with 4′,6-diamidino-2-phenylindole (DAPI). Digital images were captured using a Zeiss Axioscop epifluorescence microscope equipped with a cooled CCD camera (Photometrics, Tucson, AZ, USA). Hybridisation signals on 200 intact, well preserved, and non-overlapping nuclei were evaluated by at

least two independent observers. Two distinct experiments were performed for each case. After performing FISH experiments on different normal endometrial tissues as controls (data not shown), we defined the presence of numerical chromosomal abnormalities (referred to as aneuploidy) when more than 15% of cancer cells had three or more copies of specific chromosomal signals.

2.3. Molecular analysis

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). These loci were chosen on the basis of the suggestions of a panel of experts, who indicated this set of markers as the best tool to identify cancers with MSI [31]. All primer sequences were as reported in Genome DataBase (GDB, at: http://www.gdb.org). Presence of MSI (referred to as MSI+) was defined in each patient by detection of at least two unstable (due to deletions or insertions) microsatellite markers in tumour DNA when compared with normal DNA [32]. For each marker analysis, polymerase chain reaction (PCR) was carried out as previously described in Ref. [33] using fluorescence-labelled primers. Separation and analysis of the PCR products were performed by gel-electrophoresis on a 377 automated DNA Sequencer (Applied Biosystems, Foster City, CA, USA).

To evaluate the expression status of the MLH1 and MSH2 proteins, immunohistochemistry (IHC) was performed on 2-μm sections of formalin-fixed, paraffin-embedded tissues obtained using standard procedures [34]. Staining was evaluated semiquantitatively, using normal epithelial cells or the centres of lymphoid follicles as internal controls. The intensity of the nuclear staining was used to classify the tumour samples as positive (strong, moderate or diffusely weak staining; referred to as IHC+) or negative (absent or focally weak staining; referred to as IHC−) for MLH1/MSH2 protein expression. IHC scoring was performed by at least two investigators.

2.4. Statistical analysis

Using Pearson's Chi-square test, presence of MSI and/or aneuploidy were assessed for association with different clinical and pathological parameters: stage of disease; grading; disease-free survival (DFS) and overall survival (OS). The exact coefficient for the sample proportion analysis was performed to determine all significant parameters (below the 0.05 level). All analyses were performed with the statistical package Statistical Package for the Social Sciences (SPSS)/7.5 for Windows.

3. Results

3.1. Analysis of archival tissues

Paired normal and tumour paraffin-embedded tissues from 86 EC patients were analysed for both genetic instability and cytogenetically-detected variation in chromosome copy number. Patients originated mainly from North Sardinia and underwent interviews for familial recurrence of the disease. Among the interviewed patients, presence of the HNPCC syndrome [30] has been excluded and no significant evidence of tumours in first- and second-degree relatives was observed. Clinicopathological characteristics of EC patients are listed in Table 1. The proportion of patients (47; 55%) presenting with disease confined to the corpus uteri (FIGO stages IA–IC) was slightly higher than that of patients (39; 45%) with disease extending beyond the corpus uteri (FIGO stages II and III) (Table 1). Endometrioid adenocarcinoma was the most prevalent histological variant (67; 78%) (Table 1).

Gain of chromosomes (referred to as aneuploidy) was assessed by fluorescence *in situ* hybridisation (FISH) on paraffin-embedded nuclei from the EC tissues. The most frequently observed cytogenetic anomalies in tumour cells were trisomies. Conversely, no karyotypic alteration was found in cells from normal tissues surrounding the tumours. In Fig. 1 some examples of the FISH results are represented, showing EC cases with a normal number of chromosomes 1 and 10 (Fig. 1a and b,

Table 1
Patients' characteristics: staging was according to the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO)

Characteristics	Number of patients (%)
Total analysed	86 (100)
Age	
Median (years) range	63 (41–87)
⩽50	9 (10)
50–59	23 (27)
>60	54 (63)
FIGO Stage	
IA	5 (6)
IB	29 (34)
IC	13 (15)
II	11 (13)
III	28 (33)
Histology	
Endometrioid	67 (78)
Adenocarcinoma	13 (15)
Serus carcinoma	6 (7)
Grading	
G1	18 (21)
G2	49 (57)
G3	19 (22)

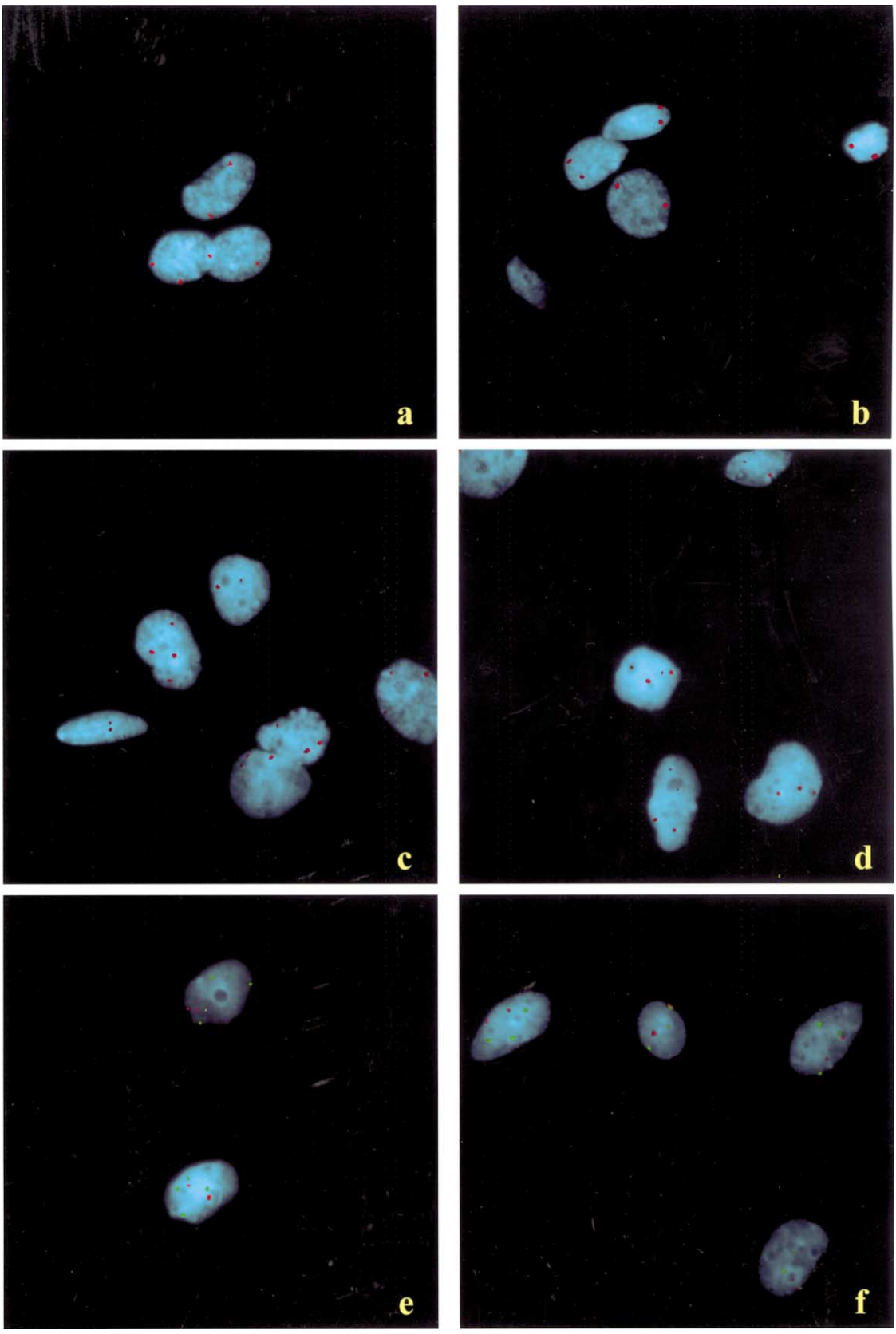

Fig. 1. Typical examples of results from fluorescence *in situ* hybridisation (FISH) analysis. Nuclei extracted from paraffin-embedded endometrial cancer (EC) tissues after hybridisation with probes specific for chromosome 1 (*a* and *c*) and chromosome 10 (*b* and *d*). Double-colour FISH with probes specific for chromosomes 10 (green signals) and chromosome 18 (red signals) (*e* and *f*).

respectively) or trisomy/tetrasomy of chromosome 1 (Fig. 1c) and chromosome 10 (Fig. 1d). Further investigation on a subset of EC cases using the two-colour hybridisation approach gave overlapping results, thus confirming the sensitivity and specificity of the FISH analysis. In Fig. 1e and f, tumours showing multiple copies of chromosome 10 (in green) and a normal copy number of the control chromosome 18 (in red) are presented.

In our series, 38 (44%) EC tumours exhibited aneuploidy and are listed in Table 2. In 13 (15%) cases, gain of copies was also observed for chromosome 18 that was used as control, indicating the presence of more complex karyotypic anomalies (indeed, aneuploidy of such a chromosome was always associated with numerical abnormalities of chromosome 1 or chromosome 10 or, commonly, both; see Table 2).

Table 2
Endometrial cancers showing aneuploidy. Results of fluorecence *in situ* hybridisation (FISH) analysis for all three chromosomes are given for each tumour sample. Presence of microsatellite instability (MSI)+ phenotype as well as results from immunohistochemistry (IHC) are also indicated

Tumour sample	Numerical abnormality			MSI+	IHC	
	Chrom. 1	Chrom. 10	Chrom. 18		MLH1	MSH2
1	+	+	–	NO	+	+
2	–	+	–	NO	+	+
3	+	+	–	NO	+	+
4	+	+	+	NO	+	+
5	+	+	+	n.t.	+	+
6	+	–	–	NO	+	+
9	+	+	–	n.t.	n.t.	n.t.
10	+	+	–	YES	–	+
12	+	–	–	NO	+	+
13	+	–	–	n.t.	n.t.	n.t.
14	+	+	+	NO	n.t.	n.t.
15	+	+	+	NO	+	+
16	–	+	–	n.t.	n.t.	n.t.
21	+	–	–	YES	–	+
23	+	–	+	n.t.	n.t.	n.t.
24	–	+	+	NO	n.t.	n.t.
25	+	+	–	n.t.	n.t.	n.t.
26	–	+	–	YES	–	+
27	+	+	–	NO	n.t.	n.t.
28	–	+	–	NO	n.t.	n.t.
33	+	+	–	YES	–	+
34	+	–	–	YES	n.t.	n.t.
35	+	+	+	NO	+	+
36	+	+	+	NO	+	+
37	–	+	–	n.t.	n.t.	n.t.
39	–	+	–	NO	+	+
57	+	–	–	NO	n.t.	n.t.
83	–	+	–	YES	–	+
93	–	+	–	NO	+	+
94	–	+	–	NO	n.t.	n.t.
95	+	+	–	YES	+	+
99	+	+	+	YES	+	+
128	+	+	+	NO	+	+
137	+	–	–	YES	–	+
145	+	+	+	YES	+	+
147	+	+	+	NO	+	+
153	+	+	+	NO	+	+
161	–	+	–	NO	+	+

n.t., not tested; Chrom, chromosome.

Comparison of amplified DNAs from tumour and corresponding normal tissues allowed us to identify the genetic instability as an electrophoretic mobility shift due to contraction or expansion of microsatellite repeats, with consequent changes in microsatellite length (as previously reported in Refs. [17,33]). Tumours were classified as MSI+ when at least two out of the five markers used displayed evidence of mutant alleles in the tumour DNA compared with the corresponding normal tissue DNA. To avoid any PCR-based artifacts, unclear or ambiguous results were confirmed in replicate experiments.

Twenty-four (32%) out of 74 tumours analysed were found to be MSI+ (Table 3). Taking into consideration the results from the FISH analysis, we classified all of the EC cases according to the presence of genome-wide MSI. Among the 38 cases showing aneuploidy and with the exception of seven tumours in which no result from the microsatellite analysis was obtained, the majority of analysed cases (21/31; 68%) were classified as stable (MSI−) tumours, with approximately one third of them (10/31; 32%) exhibiting a MSI+ phenotype (Table 3). In addition, evaluation of both MLH1 and MSH2 protein expressions by IHC in 24 neoplastic tissues from this group revealed that 6/9 (67%) MSI+ tumours presented a MLH1-negative nuclear staining (all of them were completely negative), whereas no loss of MLH1 expression was observed in the remaining 15 MSI−EC cases (Tables 2 and 3). All of the IHC-analysed EC tissues presented a normal expression of the *MSH2* gene product (Tables 2 and 3).

When we considered the EC cases without aneuploidy, an identical prevalence of genetic instability was found [14 (33%) MSI+ tumours out of 43 ECs in which results from the microsatellite analysis were obtained], indicating no difference in the MSI frequency among the two groups (with and without such cytogenetic alterations; Table 3). As a confirmation of this, aneuploidy was

Table 3
Comparison between aneuploidy and microsatellite instability; results from immunohistochemistry (IHC) analysis for MLH1 and MSH2 proteins are also indicated

Characteristics (patients)	MSI+ (%)	MSI− (%)
Aneuploidy+ (31)	10 (32)	21 (68)
	6/9 MLH1−	15/15 MLH1+
	9/9 MSH2+	15/15 MSH2+
Aneuploidy− (43)	14 (33)	29 (67)
	8/13 MLH1−	6/6 MLH1+
	13/13 MSH2+	6/6 MSH2+
Total (74)	24 (32)	506 (68)
	14/22 MLH1−	21/21 MLH1+
	22/22 MSH2+	21/21 MSH2+

Table 4
Correlation between aneuploidy (a) or microsatellite instability (MSI) (b) and both the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage of disease and the tumour grading

(a)

Characteristics (patients) 86 patients total	Aneuploidy+ (%) 38 patients	Aneuploidy− (%) 48 patients
FIGO stage		
I ($n=47$)	23 (49)	24 (51)
II–III ($n=39$)	15 (38)	24 (62)
II ($n=11$)	4 (36)	7 (64)
III ($n=28$)	11 (39)	17 (61)
Grading		
G1 ($n=18$)	7 (39)	11 (61)
G2 ($n=49$)	22 (45)	27 (55)
G3 ($n=19$)	9 (47)	10 (53)

(b)

Characteristics (patients) 74 patients total	MSI+ (%) 24 patients	MSI− (%) 50 patients
FIGO stage		
I ($n=39$)	9 (23)	30 (77)
II–III ($n=35$)	15 (43)	20 (57)
II ($n=10$)	3 (30)	7 (60)
III ($n=25$)	12 (48)	13 (52)
Grading		
G1 ($n=14$)	3 (21)	11 (79)
G2 ($n=45$)	15 (33)	30 (67)
G3 ($n=15$)	6 (40)	9 (60)

found at identical rates in the two MSI subgroups [10 (42%) out of 24 MSI+ cases, and 21 (42%) out of 50 MSI− cases] (Table 3). For the cytogenetically-normal group, we also found that 8/13 (62%) MSI+ cases showed loss of the MLH1 protein, whereas all of the six IHC-analysed MSI− tumours presented a normal MLH1 expression (Table 3). Again, no cases showed altered expression of the MSH2 protein (Table 3).

In a limited number of cases, presence of instability was evaluated at either chromosome 1 (using the BAT-40 marker, mapping to the cytogenetic band 1p13) or chromosome 10 (using microsatellite markers located at 10q25-q26), as previously described in Ref. [17]. 9 (69%) out of 13 cases with instability at chromosome 10 and seven (78%) out of nine tumours with instability at chromosome 1 also presented the MSI+ phenotype, strongly suggesting that unstable markers at chromosomes 1 and 10 should be considered as expression of the presence of genome-wide instability.

3.2. Clinicopathological correlation

All genetic and cytogenetic alterations identified were statistically correlated to histopathological and clinical parameters. As shown in Table 4, aneuploidy (a) and the presence of MSI (b) were both compared with the stage of disease and tumour grading. The only significant linearity was demonstrated for the association between the presence of the MSI+ phenotype and the worsening of both the stage of disease ($P=0.012$) and histological grading ($P=0.034$) (Table 4).

Table 5
Correlation between disease stage or molecular alterations and clinical outcome. Median values (indicated as months) of both disease-free survival (DFS) and overall survival (OS) are calculated for (a) patients grouped according to the Fédération Internationale de Gynécologie et d'Obstétrique (FIGO) stage of disease; or (b) cases with or without aneuploidy; (c) cases with or without microsatellite instability (MSI)

(a)

FIGO Stage (patients)	DFS (months)	OS (months)	*P* value
I (41)	41	44	<0.001
II+III (37)	27	28	

(b)

Characteristics (patients)	DFS (months)	OS (months)	*P* value
Aneuploidy+ (35)	32	35	>0.1
Aneuploidy−(43)	33	34	

(c)

Characteristics (patients)	DFS (months)	OS (months)	*P* value
MSI+ (22)	30	33	>0.05
MSI−(47)	33	35	

After a clinical follow-up over a median period of 49 months (range 12–87 months), no genetic or cytogenetic alteration was correlated to DFS or OS. In an univariate analysis, only the stage of the disease showed a significant association with DFS and OS (Table 5a), whereas no significant difference was observed for DFS and OS of the EC patients among the four groups (MSI+, MSI−, aneuploidy+ and aneuploidy− (Table 5, b and c).

4. Discussion

In this study, we performed a cytogenetic and molecular evaluation of sporadic ECs in order to better investigate the role of both karyotypic alterations and genomic instability in such a disease. Therefore, we evaluated the possibility of the existence of positive or negative correlations between MSI and specific chromosomal changes.

Using a reference panel of five polymorphic markers (two mononucleotide repeats and three dinucleotide repeats), DNA from paraffin-embedded tissues of sporadic EC patients was collected and investigated for genome-wide MSI. As suggested by different studies [31,32], alterations in two or more locus markers are able to identify tumours with high MSI (also referred to as a MSI+ phenotype). This instability can be revealed through the shift in the electrophoretic mobility of the analysed fragments, which is due to a change in the number of repeat units. PCR-based screening with these markers revealed the presence of this genetic alteration in one third of the analysed EC cases (24/74; 32%).

A large number of studies have documented MSI at various rates in many sporadic tumour types [35–37], suggesting that the presence of MSI may be a marker of a tendency for replication errors in human cancers [27,37]. This phenomenon has been documented in colorectal tumours of patients affected by HNPCC syndrome, where the existence of a nonfunctional mechanism of DNA repair (particularly, germline mutations of the mismatch repair genes *MLH1* and *MSH2*) has been observed [18,19,37]. In our series, IHC data, using both anti-MLH1 and anti-MSH2 antibodies on 22 MSI+ EC tumours, revealed that presence of MSI was correlated with the inactivation of *MLH1* gene expression (14 staining-negative cases; 64%), whereas the MSH2 protein was found to be normally expressed in all of the MSI+ tumours analysed (see Table 3). Moreover, 21 EC cases classified as stable (MSI−) and used as negative controls for IHC presented a normal expression of both the *MLH1* and *MSH2* gene products, confirming the good sensitivity of the microsatellite analysis in detecting genetic instability. Identification of a larger group of sporadic ECs with the MSI+ phenotype is needed to confirm that inactivating mechanisms of MLH1 play a major pathogenetic role in sporadic EC.

Similarly, a numerical chromosomal alteration or aneuploidy was detected by FISH analysis on archival tumour tissues using the protocol we previously defined in Ref. [26]. As summarised in Table 4, no significant differences in patients' distribution according to the stage of disease or grading of tumour differentiation were observed between the two groups, with a diploid or aneuploid cell population. Conversely, a significantly higher incidence of MSI was observed in FIGO stage III and in more undifferentiated tumours (see also Table 4), suggesting that genetic instability may become particularly evident in advanced stage tumours due to the progressive accumulation of errors.

Although the number of tumours undergoing such analyses could be increased, our findings seem to indicate that MSI and numerical chromosomal abnormalities may represent two unrelated phenomena in EC (MSI and aneuploidy were independently exhibited in our series; see Table 3), probably due to different pathogenetic mechanisms (as also previously suggested in Refs. [25,27]). In colorectal cancer, an inverse relationship between karyotypic alterations and MSI has been described (cancers showing MSI are, in general, diploid and exhibit normal rates of gross chromosomal changes, whereas stable tumours are usually aneuploid and exhibit increased rates of chromosomal changes) [27,38]. While a deficit in mismatch repair with a subsequent increase of the replication error rate and a sequential accumulation of genetic mutations may be the causative mechanism in tumours (including EC) with MSI [39,40], alterations affecting the molecular machinery that monitors the proper progression of the cell cycle seem responsible for the presence of some recurrent karyotypic abnormalities [27,41,42]. Therefore, one could speculate that 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, whereas near-diploid karyotypes with few or single structural or numerical anomalies may be present as a specific feature in an initial stage of the neoplasia (in contrast to the complex karyotypic anomalies with deeply heterogeneous structural and numerical alterations, which may possibly be related to advanced stages of the disease [24,25]).

Considering the correlations with clinical parameters, only stage of disease was found to be predictive as a prognostic factor. Although the MSI+ phenotype was significantly associated with both the disease stage and the histological grading, no differences in DFS and OS were observed in the groups of EC patients with or without MSI (see Tables 4 and 5). It is likely that other factors interact in determining the tumour behaviour and clinical outcome. In our series, EC patients presented with quite a heterogeneous clinical history (particularly, in terms of the therapeutical approaches) and this could account for the similar prognosis found in the two groups (with and without the MSI+ phenotype). As a confirmation of this hypothesis, results on patients with colorectal carcinoma have indicated that better survivals are observed only in early-stage MSI+ cases receiving chemotherapy (survivals among untreated patients were found to be similar in the MSI+ and MSI− groups) [43–44].

The present study suggests that both specific alterations in chromosome number and MSI seem to be associated with different tumorigenic pathways, but not to possess any significant prognostic role. However, a larger group of patients and further analyses are needed to confirm these findings in sporadic EC.

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