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Microsatellite instability predicts clinical outcome in radiation treated endometrioid endometrial cancer patients

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Abstract

Purpose—Microsatellite instability (MSI) is the hallmark of cancer with DNA mismatch repair (MMR) deficiency and underlies 20–30% of endometrial cancer. Some *in vitro* studies suggest that radiation effects are modulated by the MMR system, however, little is known about the relationship between MSI and radiation response. The aim of this study was to elucidate whether MSI predicts clinical outcome in radiation treated endometrioid endometrial cancer (EEC).

Methods—We have studied a consecutive series of 93 patients with EEC treated with extrafacial hysterectomy and postoperative radiotherapy. The median clinical follow up of patients was of 138 months, with a maximum of 232 months. Five quasimonomorphic mononucleotide markers (BAT-25, BAT-26, NR21, NR24 and NR27) were used for MSI classification.

Results—Twenty five patients (22%) were classified as MSI. Either in the whole series and in early stages (I and II), univariate analysis showed a significant association between MSI and a poorer 10-years local disease free (LDFS), disease free (DFS), and cancer-specific survival (CSS). In multivariate analysis, MSI was excluded from the final regression model in the whole series, but in early stages, MSI provided additional significant predictive information independent of traditional prognostic and predictive factors (age, stage, grade and vascular invasion) for DFS (HR, 3.25, 95%

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Conflicts of Interest Notification

The authors state that any actual or potential conflicts of interest do not exist

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CI, 1.01–10.49; $P=0.048$) and CSS (HR, 4.20; 95% IC, 1.23–14.35; $P=0.022$), and was marginally significant for LDFS (HR, 3.54; 95% IC, 0.93–13.46; $P=0.064$).

Conclusions—Our results suggest that MSI may predict radiotherapy response in early stage EEC.

Keywords

Microsatellite instability (MSI); radiotherapy; endometrioid endometrial carcinoma (EEC); outcome

INTRODUCTION

Tumors with microsatellite instability (MSI) are characterized by a massive instability in simple repeated sequences (microsatellites) caused by defects in the DNA mismatch repair (MMR) system (1). In endometrial cancer (EC), MSI is present in 20%–30% of tumors and is associated with endometrioid histology (2). MMR impairment in EC is mainly caused by silencing of *MLH1* associated with promoter hypermethylation (3). Data regarding the clinicopathological and survival impact of MSI in EC are controversial and remain unclear (2,4–6). Studies *in vitro* have shown the involvement of the MMR system in cell death signaling after sufficiently toxic DNA damage, and that MMR-deficient cells are relatively tolerant to DNA damage caused by several agents (7). Little is known about the clinical response of MMR-deficient patients to radiotherapy, and only few studies on rectal cancer treated with preoperative chemoradiation have been published (8–10). In EC, external pelvic radiotherapy and/or vaginal brachytherapy is used postoperatively for patients with tumor characteristics predicting high risk of local recurrence and poor prognosis. Here we analyze whether MSI has a predictive role in radiation therapy response in endometrioid EC (EEC).

METHODS AND MATERIALS

Patients

We retrospectively studied 93 consecutive patients with localized EEC, diagnosed and treated with given informed consent between 1990 and 1999 at the Department of Obstetrics and Gynecology of the Complejo Hospitalario Universitario Insular-Materno Infantil de Gran Canaria. The study was approved by the correspondent Research and Ethics Committee. All patients underwent exploratory laparotomy, extrafacial hysterectomy and bilateral salpingo-oophorectomy. Postoperative radiotherapy was administered at the Department of Radiation Oncology of the Hospital Universitario de Gran Canaria Dr. Negrín. In all cases radiation was given by a four field technique up to a mean dose of 50.44 ± 2.41 Gy in 1.8–2 Gy fractions, and all but 5 patients received brachytherapy. None of the patients in our series received preoperative radiation or chemotherapy. All patients were Caucasians, and the median age at diagnosis was 65 years (range: 30–90); 66 tumors were well ($n=29$) or moderately ($n=37$) differentiated, and 58 patients were classified at stage I (Table 1). Among those patients still alive at the end of the follow-up period (Jun 2009), the median follow-up time was 138 months (range, 16–232 months).

DNA preparation and MSI classification

Tumor specimens were promptly frozen in liquid nitrogen after surgery and kept at -70°C until needed. The tissue was mechanically disrupted and the DNA was extracted using standard SDS-proteinase K-phenol procedure. A similar procedure was used for DNA extraction from available matching blood.

MSI tumor determination and classification was performed by using five recommended quasimonomorphic mononucleotide markers (BAT-25, BAT-26, NR21, NR24 and NR27)

according to published conditions and criteria (11). The markers were amplified in pentaplex PCR and run in an ABI prism 3100 (Applied Biosystems).

Statistical analysis

Body mass index and age were considered continuous and the other variables as categorical. The relationship between MSI and demographic/clinicopathologic variables was performed using the χ^2 and Fisher's exact nonparametric tests (based upon sample size) and *t* tests. Disease free survival (DFS) was defined as the time from surgery to recurrence. Local disease free survival (LDFS) was defined as the time from surgery to local cancer recurrence. Patients with residual disease after surgery were excluded from LDFS and DFS analysis. Cancer specific survival (CSS) was defined as the time from surgery to the date of death from cancer. Survival curves were constructed using the Kaplan-Meier method and then compared using the log-rank test. Univariate and multivariate Cox proportional hazards models were fitted to check the possible effects of the covariates on CSS, DFS and LDFS. All analyses were two sided, and significance was set at a *P* value of 0.05. SPSS statistical package (v. 15.0) was used.

RESULTS

Evidence of MSI was identified in 20 cases (22%). Associations between tumors without (MSS) and with MSI and clinicopathologic characteristics are shown in Table 1. The MSI phenotype was associated with advanced stage (*P*=0.04) and vascular invasion (*P*=0.009).

During the follow-up period, local recurrence, recurrence of the disease, and death from the disease were observed in 14 (15%), 27 (29%), and 23 (25%) of the 93 patients respectively. In the univariate analysis, older age, advanced stage, higher grade and vascular invasion were significantly associated with poor DFS and CSS (Table 2). The same associations were found for LDFS, except for tumor grade. The presence of MSI was associated with worse 10-year LDFS (91.1% for MSS vs. 62.9% for MSI tumors, *P*=0.003), DFS (75.9% for MSS vs. 53.8% for MSI tumors, *P*=0.03) and CSS (82.8% for MSS vs. 58.7% for MSI tumors, *P*=0.044) (Fig. 1).

To study the statistically independent predictive value of MSI, a basic multivariate model was designed by the step-down procedure after including the traditional prognostic and predictive factors that approached significance in the univariate analysis (age, stage, grade and vascular invasion). In the whole series, MSI did not contribute to the final model (Table 3A). However, in early stages (I and II), MSI provided significant additional predictive information for DFS (HR, 3.25, 95%CI, 1.01–10.49; *P*=0.048) and CSS (HR, 4.20; 95%IC, 1.23–14.35; *P*=0.022), and was marginally significant for LDFS (HR, 3.54; 95%IC, 0.93–13.46; *P*=0.064) (Table 3B).

DISCUSSION

The highly conserved DNA MMR proteins contribute to DNA replication fidelity by removing insertion/deletion loops and correcting single base mismatches that escape polymerase proofreading. However, evidence is accumulating about the role for MMR in cellular response to some forms of exogenous DNA damage beyond that of DNA repair (7). Some *in vitro* studies have reported that MMR-deficient cells are slightly more resistant to ionizing radiation (IR) than their MMR-proficient counterparts (7). It was also suggested that, after IR, MMR proteins are required for efficient G2-phase cell cycle arrest, being able to induce apoptosis in a p53-independent pathway (12).

Only few studies in rectal cancer have analyzed the clinical response of MMR-deficient patients to radiotherapy, and generally no influence was found (8–10). Similarly, according to our results MSI did not have independent predictive value in the whole series. However, our data also

showed that MSI had significant predictive value independent of the traditional factors, for DFS and CSS, and was marginally significant for LDFS when considering only patients bearing early stage tumors (stages I/II). Therefore, it is possible that the very poor prognosis of stage III tumors, despite of the postoperative radiotherapy treatment, could be acting as a confounding factor when determining the predictive role of MSI in EEC.

Thus, in some way, our results agree to some previous *in vitro* studies (7), although, the mechanism by which the MMR complex may influence damage response to IR is not yet clear. IR generates a large number of lesions in DNA, including double-strand breaks (DSBs), single-strand breaks, and a wide variety of base and sugar damages. It is believed that the MMR-dependent apoptosis is likely caused by the binding of MMR complexes to 8-oxoguanine generated by IR and happens in cells with excessive unreparable DNA damage (12). However, the predominant toxic DNA lesions in IR-exposed cells are DSBs. These lesions can be repaired by either non-homologous end joining (NHEJ), which is often mutagenic due to the loss of sequence information, or by homologous recombination repair (HRR), which is not mutagenic if identical sequence information is used to repair the broken substrate. A role for MLH1 in modulation of error-prone NHEJ has been recently proposed in a study with mice embryonic fibroblasts (13). Although the reason for this observation has not been determined, it was suggested that it could be by inhibiting the annealing of DNA ends containing noncomplementary base pairs or by promoting the annealing of microhomologies (13). It has also been reported that IR induces mitotic recombination and an elevated mutagenic response in *MLH1* null mouse cells (14). Mitotic recombination is a type of HRR that involves crossover events between homologous autosomal chromosomes and that frequently converts heterozygous deficient loci to homozygous deficiency after replication has occurred. MMR deficient cells present a high spontaneous rate of small mutational events. Therefore, it was suggested that an induction of mitotic recombination, after IR exposure, may create large numbers of loci that are heterozygous deficient in MSI tumors cells, which could markedly increase their malignant potential (14). In support of these findings, a very recent work in human cell lines sustains the implication of MLH1 in HRR error prone processes induced by the presence of DNA DSBs in a MMR-independent manner (15). Therefore, absence of MLH1 protein through promoter hypermethylation, which is the main cause of MSI in sporadic EC, could drive to the accumulation of DNA aberrations increasing tumor cell malignancy after irradiation.

In conclusion our results suggest a possible predictive role of MSI in radiation response in early stage EEC. Considering the limited size of our series, the results must be considered as preliminary data, and further studies should be carried out for confirmation.

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Figure 1. (a) Local Disease Free Survival, (b) Disease Free Survival and (c) Cancer Specific Survival according to tumor microsatellite status in patients with primary endometrioid endometrial cancer. MSI, microsatellite instability; MSS microsatellite stability.

Table 1**Patient Characteristics and Distribution of Microsatellite Instability**

Characteristic	Whole series No. (%)	MSI No. (%)	MSS No. (%)	P
All cases	93	20 (22)	73 (78)	
Age				ns
Mean	64.63	66.2	64.21	
Standard deviation	9.23	10.61	8.85	
Body mass index				ns
Mean	30.9	29.3	31.3	
Standard deviation	5.2	5.8	5.0	
Menopause				ns
No	9 (10)	3 (15)	6 (8)	
Yes	84 (90)	17 (85)	67 (92)	
Hypertension				ns
No	51 (55)	11 (55)	40 (55)	
Yes	42 (45)	9 (45)	33 (45)	
Diabetes				ns
No	67 (72)	11 (55)	56 (77)	
Yes	26 (28)	9 (45)	17 (23)	
Stage				0.04
I	58 (62)	8 (40)	50 (68)	
II	21 (23)	6 (30)	15 (21)	
III	14 (15)	6 (30)	8 (11)	
Grade				ns
1	29 (31)	4 (20)	25 (34)	
2	37 (40)	8 (40)	29 (40)	
3	27 (29)	8 (40)	19 (26)	
Myometrial invasion				ns
No	53 (57)	12 (60)	41 (56)	
Yes	40 (43)	8 (40)	32 (44)	
Vascular invasion				0.009
No	57 (61)	7 (35)	50 (68)	
Yes	36 (39)	13 (65)	23 (32)	

MSI, microsatellite instability; MSS, microsatellite stability; ns, non significant

Table 2
Univariate Analysis for Local Disease Free, Disease Free and Cancer Specific Survival

Characteristic	LDFS			DFS			CSS		
	HR (95% CI)	P		HR (95% CI)	P		HR (95% CI)	P	
Age	1.11 (1.04–1.20)	0.004		1.09 (1.03–1.15)	0.002		1.09 (1.04–1.15)	0.001	
Body mass index		ns			ns			ns	
Menopause		ns			ns			ns	
Hypertension		ns			ns			ns	
Diabetes		ns			ns			ns	
Stage		0.001			0.000			0.002	
I*									
II	5.87 (1.46–23.35)	0.013			ns			ns	
III	9.45 (2.24–39.92)	0.020		6.67 (2.75–16.20)	0.000		5.60 (2.09–15.00)	0.001	
Grade		ns			0.030			0.025	
I*									
2					ns			ns	
3				2.69 (1.05–6.84)	0.039		3.31 (1.15–9.57)	0.027	
Myometrial invasion		ns			ns			ns	
Vascular invasion									
No*									
Yes	3.48 (1.16–10.42)	0.018		3.11 (1.44–6.72)	0.002		4.06 (1.71–9.64)	0.001	
MSI									
No*									
Yes	4.22 (1.48–12.1)	0.003		2.38 (1.06–5.32)	0.030		2.37 (1.00–5.61)	0.044	

LDFS, local disease free survival; DFS, disease free survival; CSS, cancer specific survival; HR, hazard ratio; ns, non significant; MSI, microsatellite instability; *, reference category.

Table 3

Multivariate analysis for local disease free, disease free and cancer specific survival in the whole series (A) and considering only early stages (I and II) (B)

WHOLE SERIES						
Characteristic	LDFS		DFS		CSS	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
Age	1.010 (1.03–1.19)	0.007	1.09 (1.03–1.14)	0.002	1.08 (1.02–1.14)	0.004
Stage		0.009		0.001		0.030
I *						
II	6.90 (1.50–31.82)	0.013	2.35 (0.81–6.82)	0.115	2.85 (0.91–8.96)	0.072
III	9.90 (2.11–46.45)	0.004	7.58 (2.72–21.08)	0.000	3.86 (1.35–11.04)	0.012
Grade		0.049		0.029		0.069
I *						
2	1.84 (0.31–10.76)	0.501	1.26 (0.41–3.89)	0.690	1.11 (0.32–3.87)	0.865
3	6.09 (1.20–30.78)	0.029	3.33 (1.25–8.92)	0.016	3.07 (1.03–9.13)	0.044
Vascular invasion						
No *						
Yes	1.89 (0.60–5.97)	0.281	1.93 (0.85–4.42)	0.119	2.79 (1.12–6.96)	0.028
MSI						
No *						
Yes	1.45 (0.42–4.99)	0.559	0.98 (0.38–2.55)	0.963	0.83 (0.29–2.36)	0.728
EARLY STAGES (I&II)						
Characteristic	LDFS		DFS		CSS	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
Age	1.11 (1.01–1.22)	0.029	1.12 (1.04–1.2)	0.003	1.13 (1.04–1.22)	0.004
Grade				0.181		0.133
I *						
2			0.4 (0.11–1.52)	0.179	0.34 (0.08–1.62)	0.185

EARLY STAGES (I&II)

(B)

Characteristic	LDFS		DFS		CSS	
	HR (95%CI)	P	HR (95%CI)	P	HR (95%CI)	P
3			1.20 (0.36–3.94)	0.769	1.38 (0.38–4.95)	0.626
Vascular invasion						
No *						
Yes	3.96 (0.95–16.42)	0.058	2.70 (1.00–7.31)	0.050	3.74 (1.26–11.12)	0.018
MSI						
No *						
Yes	3.54 (0.93–13.46)	0.064	3.25 (1.01–10.49)	0.048	4.20 (1.23–14.35)	0.022

LDFS, local disease free survival; DFS, disease free survival; CSS, cancer specific survival; HR, hazard ratio; MSI, microsatellite instability;
*, reference category.