Allelic imbalance in *hMLH1* or *BRCA2* loci associated with response of cervical and endometrial cancer to radiotherapy

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Abstract. Effectiveness of radiotherapy is influenced by several genetic properties of the targeted cells. The aim of this study was the identification of prognostic indicators of tumor response to radiation in cervical and endometrial cancer. Using microsatellite DNA analysis, we investigated 31 markers, located on 1p, 2p, 2q, 3p, 9p, 9q, 13q, 17p and 17q for genomic alterations in 37 cervical and 21 endometrial cancer cases, with complete follow-up data. Genetic alterations of the initial tumor genotypes were observed after radiation in 86.5% of cervical and 81.0% of endometrial cases. Reversions to the original normal genotype were observed in 40.5 and 28.6% respectively, predominantly in cured patients rather than in recurred cases. Survival curves by the Kaplan-Meier method showed a worse prognosis for cervical cancer patients whose tumors harbor allelic imbalance (AI) on 3p or 13q, and for endometrial cancer patients whose tumors harbor AI on 13q. Our data suggest a possible association of the *hMLH1* or *BRCA2* genes, implicated in distinct DNA repair pathways and located on 3p and 13q respectively, with response of cervical and endometrial cancer to radiotherapy. Moreover, microsatellite DNA analysis before and after radiation treatment could be used as a marker of the clinical outcome of patients.

Introduction

Radiotherapy is the most effective conservative treatment for cervical cancer while in the case of endometrial cancer it is reserved for women for whom the risk of surgery is high. The main scope of radiation treatment is to sterilize the disease in the cervix or endometrium, adjacent tissues and regional lymph nodes in the pelvis because of DNA damage and cell death linked to mitosis (1). In addition, radiation produces a variety of other cellular perturbations, such as effects on growth factors and signal-transduction pathways, apoptosis, and changes in the regulation of the cell cycle (2).

However, the effectiveness of radiotherapy is restricted, since tumors exhibit a wide diversity of responses to such treatment and the appearance of resistant subpopulations upon relapse of an originally responsive malignancy could occur. The ability to predict which patients might fail radiation treatment, and who could benefit from alternative therapeutic protocols, is important to improve the survival rates for women with cervical and endometrial cancer. The stage of disease, the grade of tumor, the cell type, and the depth of stromal invasion have been proposed as markers for the response to radiotherapy (3).

Genomic instability is a common feature of neoplastic cells, due to rapid proliferation or elevated mutational rate. The highly polymorphic microsatellite DNA has been extensively used for genetic analysis in various diseases, including neoplasias, as chromosome mapping tool, as means for the evaluation of DNA integrity or for the identification of novel genes implicated to specific diseases (4,5). Detection of an asymmetric intensity ratio between two alleles at a locus, as compared to the profile of normal cells, is described as allelic imbalance (AI) for the tumor in question. AI may reflect the complete loss of one allele at a constitutional heterozygous locus that is masked by the presence of normal cells, by tumor heterogeneity, or by non-clonal loss. Increased DNA copy number will also reveal an AI pattern (6). Such alterations comprise a pathway for the full inactivation or altered expression of genes close to the affected microsatellite marker (7). Hence, these markers represent molecular tools for predicting the clinical behavior of tumors.

Previous studies have found associations of genetic alterations on 6p21.2 and 17p chromosomal regions with response of cervical cancer after radiotherapy (8,9). In this study, we investigated the biological significance of genetic alterations on 1p, 2p, 2q, 3p, 9p, 9q, 13q, 17p and 17q in biopsy specimens from patients with cervical or endometrial cancer obtained before the initiation and 3 months after the termination of radiation treatment.

Patients and methods

Patient population. Over the period of March 1997 to May 1999, 37 patients with histological proven cervical and 21

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Table I. Patients and tumor profiles.

Characteristic	Cervical	Endometrial
Total no. of patients	37	21
Mean age ± SD (year)	57.3±9.0	58.0±10.5
FIGO[a] stage		
I	2	1
II	17	11
III	13	7
IV	5	2
Differentiation		
Mild (grade I)	10	5
Moderate (grade II)	13	7
Poor (grade III)	14	9

[a]FIGO, the International Federation of Gynecology and Obstetrics classification.

endometrial cancer were treated with radiotherapy at University Hospital of Heraklion, Crete (Table I). Staging of the disease was performed according to the International Federation of Gynecology and Obstetrics (FIGO) classification (10,11), and the World Health Organization (12), by history and physical examination, including pelvic examination, chest roentgenography, intravenous pyelography, barium enema, cystoscopy, proctoscopy, and computerized tomography (CT) of the abdomen. The patients were followed up monthly after the completion of radiotherapy. The follow-up for the surviving patients ranged from 3 to 52 months, with a mean of 39.2 months. Tumor biopsies were performed before the initiation and 3 months after the termination of radiation treatment from the same tumor region, in order to avoid sampling of a different subpopulation of cancer cells. Corresponding venous blood samples were collected prior to radiotherapy. Tissue specimens were stored at -80°C, while venous blood in K^+/Na^+ EDTA tubes at 4°C, until DNA extraction. The University of Crete Ethics Committee approved this study and all the patients gave written informed consent.

Irradiation treatment protocol. The patients entered in the study were radiated with a combination of external beam radiotherapy and intracavity brachytherapy. The external radiation was delivered to the tumors in fractions of 1800 cGy daily, 5 days/week, while the dose of ^{192}Ir intracavity brachytherapy was administered at a high dose rate, once a week. Intracavity brachytherapy was performed in all patients, with either one or two tandem and ovoid applications, with tumor dose specified to point A (point A being 2 cm lateral to the central canal of the uterus and 2 cm up from the mucous membrane of the lateral fornix, in the axis of the uterus). The median brachytherapy dose was 4120 cGy, and the median total dose to point A from both external beam and implant contributions was 8095 cGy. The response of the tumor to the treatment was defined as follows: positive response when tumor mass was reduced ≥60% 3 months after the finish of radiation treatment; and non-response when the reduction in tumor mass was <40%.

Table II. Microsatellite DNA markers studied.

Marker	Cyto-genetic location	Hetero-zygosity	Allele size range	Optimal multiplex PCR primer concentration (nM)	Panel[a]
D1S116	1p31.2	0.65	89-101	100	A
CLN1	1p32	0.87	140-209	130	A
MYCL1	1p32	0.87	140-209	120	B
D2S123	2p22.3	0.773	197-227	210	C
D2S177	2p21	0.854	276-302	230	B
D2S147	2p13.2	0.725	126-144	150	D
D2S2182	2p23.3	0.78	234	200	B
D2S288	2p23.3	0.62	276-284	250	A
D2S2291	2p23.2	0.76	245	210	E
D2S113	2q11.1	0.777	206-230	225	A
D2S138	2q21-q33	0.67	111-125	100	B
D2S164	2q33-q37	0.83	265-303	260	F
D2S105	2q23-q35	0.69	107-125	125	C
D2S311	2q35-qter	0.81	185-207	200	F
D3S1612	3p22.3	0.69	100	110	D
D3S1478	3p21.31	0.98	109-152	150	b
D3S1611	3p24.2-p22	0.66	252-268	225	G
D3S1260	3p21.32	0.66	268	210	H
D3S1561	3p22.1	0.65	100	150	E
D9S161	9p21	0.783	119-135	125	b
IFNA	9p22	0.72	138-150	130	E
D9S270	9p21	0.71	87-101	90	F
D9S112	9q31-q34	0.884	115-135	120	F
D13S220	13q12.1	0.668	191-203	200	D
D13S290	13q12.1	0.75	241-253	260	C
D13S289	13q12.1	0.74	260-276	280	D
TP53	17p13.1	0.9	103-135	150	b
THRA1	17q11.2-q12	0.81	158-176	150	C
D17S579	17q21	0.8	111-113	120	G
D17S855	17q21	0.82	145	160	G
D17S515	17q21	0.9	107-127	130	H

[a]Panel of multiplex reactions; [b]Single-plex reaction.

DNA extraction. DNA was isolated from peripheral white blood cells and biopsy specimens using the IsoQuick Nucleic Acid Extraction kit (ORCA; Research, Inc., Bothell, WA), according to the manufacturer's instructions.

Microsatellite analysis. Thirty-one highly polymorphic microsatellite DNA markers (Research Genetics, USA), were used. The chromosomal regions 1p32-p31, 2p22-p21, 2q21-q33, 3p24.2-p22, 9p21, 9q31-q34, 13q12.3, 17p13.1 and 17q21

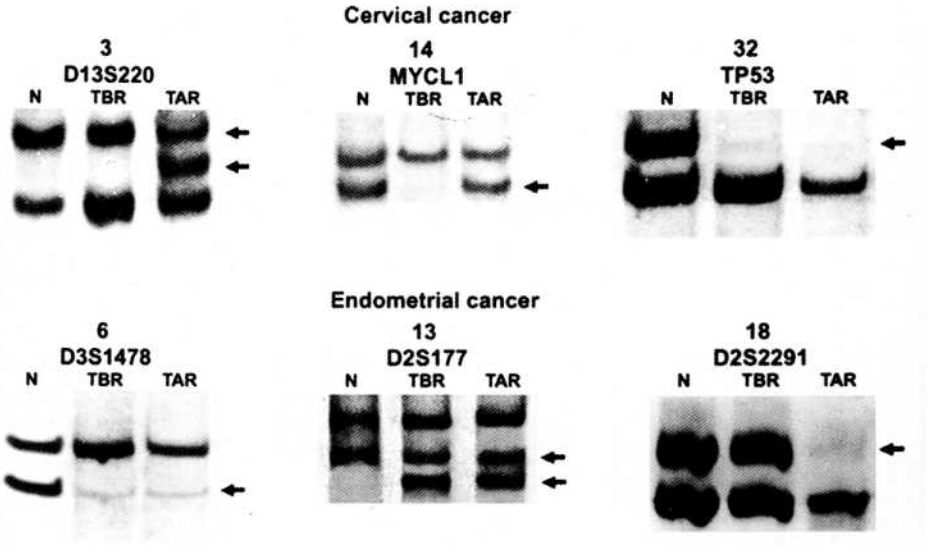

Figure 1. Representative examples of specimens exhibiting allelic imbalance and microsatellite instability. N, normal, venous blood before radiotherapy; TBR, tumor before radiotherapy; TAR, tumor after radiotherapy. The numbers indicate the sample number for cervical (upper panel) and endometrial cancer (lower panel) cases. A single arrow indicates the deleted allele in AI cases. Two arrows indicate the shift in the mobility of the affected alleles in MI cases.

were selected according to the proximity to *c-Jun*, *hMSH2*, *hPMS1*, *hMLH1*, *p16^{Ink4}*, *c-Abl*, *BRCA2*, *TP53* and *BRCA1* respectively (data from Genome Database, http://gdbwww.gdb.org). The primers were amplified in 5 panels of 4-plex, 2 panels of 3-plex, one 2-plex and 3 single-plex PCRs. To optimize multiplex reactions, different concentrations of each primer set were used (Table II). We introduced 100 ng of genomic DNA in a PCR reaction mixture containing 1X PCR buffer, 400 μM dNTPs, 2.66 mM $MgCl_2$ and 0.35 U Taq DNA polymerase (Gibco BRL, Life Technologies). Amplification parameters were the following: initial denaturation for 3 min; 30 cycles at 94°C for 30 sec, 55°C for 30 sec and 72°C for 30 sec; final extension step at 72°C for 10 min.

Digital imaging. PCR products were electrophoresed in a 10% polyacrylamide gel and silver stained. Gels were scanned on an Agfa SnapScan 1212u (Agfa-Gevaert N.V., Belgium). Integrated density [ID = (mean OD - background OD)* pixels; OD, optical density], of the bands was used as quantitative parameter and was calculated by digital imaging using the Adobe Photoshop 6.0 software (Adobe Systems Inc., USA). Allelic imbalance (AI) was scored in a heterozygous case when

$$\text{the ratio } \frac{\text{ID allele 1 (tumor DNA)/ID allele 2 (tumor DNA)}}{\text{ID allele 1 (blood DNA)/ID allele 2 (blood DNA)}} \text{ was}$$

calculated >1.49 or <0.58, with a 99.5% confidence interval, as determined from independent reproducibility experiments (13). Microsatellite instability (MI) was scored when a novel generated microsatellite allele was observed in tumor tissue-extracted DNA compared to the correspondent venous blood-extracted DNA. The analysis in AI or MI positive cases was repeated three times and the results were reproducible. Representative examples of AI and MI are shown in Fig. 1.

Statistical methods. All statistical calculations were carried out by the SPSS 8.0 (SPSS Inc., USA) program. Differences in the mean values of quantitative measurements were tested using the Student's t-test or Mann-Whitney test. The relationship between the presence or absence of genetic alterations and the response to radiotherapy was analyzed with Fisher's exact test. The overall survival curves were calculated according to the Kaplan-Meier method and differences in survival were analyzed with the log-rank test. A p-value <0.05 was considered as statistically significant.

Results

Cervical cancer. We assayed 37 cervical cancer/venous blood DNA pairs with a total of 31 microsatellite markers before the initiation of radiotherapy. The individual allelotypes for each cervical cancer patient studied are shown in Table III. Allelic imbalance (AI) was observed in 83.8% (31 of 37) of cases in at least one marker, while D3S1478 (33.3%), IFNA (32.3%), D3S1611 (29.6%), D9S270 (29.0%) and D3S1260 (28.6%) were found most frequently affected. The fractional allelic loss (FAL) value was calculated for each sample as (loci scored with allelic imbalance)/(total informative loci) (14). The mean FAL value was 0.17 and the highest was 0.41 in a stage III case, aged 51 years, who died from disease recurrence. The fractional regional loss (FRL) values were calculated for each sample as (loci on the arm with allelic imbalance)/(total informative loci on the arm) (14). An analytical presentation of FAL and FRL values for each cervical cancer case is provided in Table V. AI was found predominantly on chromosomal regions 3p (56.8%), 2q (54.1%) and 9p (45.9%) with mean FRL values 0.29, 0.18 and 0.25 respectively. No statistically significant difference was found between FAL or FRL values and clinicopathological parameters.

Microsatellite instability (MI) in at least one marker was observed in 35.1% (13 of 37) of cervical cancer specimens before radiotherapy. However, none of these cases fulfilled the criterion of the MI co-finding on at least 5 markers to be

Table III. Individual allelotypes for the cervical cancer cases before and after radiotherapy.

A

		Microsatellite DNA markers															
Pt. No.	Age	D13S116	CLN1	MYCL1	D2S123	D2S177	D2S147	D2S2182	D2S288	D2S2291	D2S113	D2S138	D2S164	D2S105	D2S311	D3S1612	D3S1478
1	50	H	H	H	AI/H	AI	H	AI/H	NI	H	H	NI	AI/H	H	H	H/MI	H
2	74	AI	AI	AI	H	NI	H	H	H	H	H	H/MI	H	AI/H	AI/H	AI	AI
3	55	H/MI	H	H	NI	H	H	H	NI	H	H	H	AI	NI	H	H	H
4	68	H	H	H	H/MI	H	H	H	H	H	H	H/MI	H	H	AI	NI	AI
5	71	H	H	H	H	H	H	H	H	H	NI	H	H	H	H	AI	H
6	52	H	H	H	H	H	NI	H/MI	NI	H	H	H	H	H	H	H	H
7	46	H	AI	AI	H	H	H	H	H	NI	H	NI	H	NI	H	H	H
8	45	H	H	H/MI	NI	H	H/MI	H	AI	H	AI	H	H	H	H	H	AI
9	75	AI	AI	H	H	H	H	H	H	H	H	AI/H	H	H	H/MI	NI	H
10	76	H	NI	H	H	H	H	H	H	H	H	H	H	AI	H	H	H
11	51	H	H	H	H	H	H	H	H	H	NI	H	H	H	H	H	H
12	46	H	H	AI	H	H	H	H/AI	H	H	H	H	H	AI/H	H	H	H
13	58	H/MI	H	H	H	H	H	H	NI	H/AI	AI	NI	H	H	H	AI	H
14	62	H	NI	AI/H	H	H	H	H	H	H	H	H	AI/H	NI	AI/H	H	H/MI
15	67	AI	AI	H	H	H	H	H	H	H	H	H	H	H	H	NI	H
16	55	H	H	H	H	H	NI	H	H	H	H	H	H	H	H	NI	H
17	56	H	H	H	H	H	H	H	H/MI	H	H	NI	H	H	H	H	AI
18	64	H	H	H	H	NI	H	H	NI	H/MI	H/MI	H	AI	AI	H	H	AI
19	56	H	AI	AI	H	H	NI	H	H	H	H	AI	H	H	H	AI	H
20	50	H	H	H	H	H	H	H/MI	NI	H	NI	AI	H	NI	AI	AI	AI
21	43	AI	H	NI	H/MI	H	H	H	NI	NI	H	H	NI	NI	NI	H	H
22	62	NI	H/MI	H	NI	H	H	NI	H	NI	AI	NI	H	H	H	NI	AI
23	57	H	H	H	NI	H	H	H	H	H	H	NI	H	NI	NI	NI	H
24	51	H/MI	NI	H	AI	H/MI	NI	AI	AI	H/MI	NI	H	H/AI	NI	AI	AI	AI
25	43	H	H	H	H	H	H	NI	H	H	H	H	NI	H	H	H	H
26	56	NI	H	H	NI	H	H	H	NI	H	NI	AI	H	AI	H	NI	H
27	62	H	H	H	H	H	H	NI	H	NI	H	H	AI	AI	NI	H	AI
28	50	AI/H	AI/H	AI	H	H	NI	H	NI	H	AI	H	H	NI	H	NI	AI/H
29	68	NI	H	H	H/MI	H	H	H	H	H	H	H	AI	H	AI	H	H
30	63	NI	H	H	NI	H	H	AI	NI	AI	H	H	H	H	H	H	H
31	54	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	AI/H
32	59	AI/H	AI/H	NI	H	MI	H	H	H	H	MI	H	H	H/MI	H	MI	AI
33	65	NI	AI	AI	H	H	H/MI	H	NI	H	H	H	H	H	MI	H	H
34	52	H	H	H	AI	AI	H	H	MI	H	H	H	H	H	H	AI	H
35	47	NI	H/MI	H	H	H	H	H	H	H	MI	H	AI	NI	AI	H	MI
36	58	NI	H	H	H	H	H	H	NI	H	H	NI	H	NI	H	NI	H
37	53	H/MI	H	NI	NI	H	H/AI	NI	H	H	H	NI	NI	NI	H	AI	H
AI (%)		20.0	23.5	20.6	10.0	5.9	0.0	9.1	8.3	3.0	13.3	13.8	20.6	23.1	21.2	29.6	33.3
MI (%)		0.0	0.0	0.0	0.0	4.0	0.0	0.0	4.0	0.0	8.0	0.0	0.0	0.0	4.0	4.0	4.0

B

		Microsatellite DNA markers															
Pt. No.	Age	D3S1611	D3S1260	D3S1561	D9S161	IFNA	D9S270	D9S112	D13S220	D13S290	D13S289	TP53	THRA1	D17S579	D17S855	D17S515	
1	50	NI	NI	AI/H	H	NI	H	H	AI/H	H	NI	H	H/MI	H	H	H	
2	74	NI	AI	H	H	H	H	NI	H	H	H	H/AI	H	H	H	H	
3	55	H	H	NI	AI	AI	NI	H	H/MI	H	H/MI	H	H	H	H	H	
4	68	NI	H	H	H	H/AI	MI	H	AI	AI	MI	H	NI	H	H	H	
5	71	AI	H	H	H	H	AI	H/AI	H/AI	MI	H	H	H	H	MI	MI	
6	52	H	NI	H	H	H	H	H	H	H	H	H	H	H	H	H	
7	46	H	AI	H	AI	NI	AI	H	H	H	H	H/AI	H	H	H/MI	H/AI	
8	45	AI	AI	NI	MI	MI	H/AI	H	MI	H/MI	H	H	H	MI	AI	H	
9	75	NI	NI	H	H	H	NI	H	H	H	H/AI	H	H/MI	H/AI	H/AI	AI	
10	76	H	H	H	H	AI/H	H	NI	H	H	H	H	H	H	H	H	
11	51	H	H	H	H	H	H	H	H	H	MI	H	H	H	H	H	
12	46	H	H	H	H	NI	H/MI	H	H	H	H	H	H	H	H	H	
13	58	H/MI	H	AI	H	AI	AI	AI	AI	H	H	H	MI	NI	H	H	
14	62	H	H	NI	AI	H	H	NI	H	H	NI	H	H	H	H	H	
15	67	NI/MI	NI	H	H	H/MI	AI	H	H	H	H	H	H	H	H	H	
16	55	H	H	H	H	H	H	H	H	H	H	H	H	H	H	H	
17	56	AI	H	NI	H	AI/H	H	NI	H	H	H	AI/H	H	H	H	H	
18	64	H	NI	H/MI	H	H	AI	H	NI	AI	AI	H	H	H	H	H	
19	56	AI	AI	H	H	AI	AI	H	H	H	H	H	H	H	H	H	
20	50	H	H	NI	H	H	H	H	AI	AI	AI	H	H	H	H	NI	
21	43	H	NI	H	H	AI/H	H	NI	H	H	H	H	H	NI	H	H	
22	62	NI/MI	NI	H/AI	MI	AI	H	H	NI	AI	AI	H	H	H	NI	H	
23	57	NI	H	H	H	H	MI/H	H	H	H	H	NI	H	H	H	H	
24	51	NI	H	NI	H	NI	NI	H	AI	H	AI	H	H/AI	H/AI	NI	AI	
25	43	H	NI	NI	H/MI	H	H	NI	NI	H/MI	H	H	H	H	H	H	
26	56	AI/H	H	AI/H	H	H	H	H	H	H	H	H	H	H/MI	H	H	
27	62	AI	NI	AI	AI	H	AI	H	NI	NI	H	AI/H	H	H	H	H	
28	50	H	AI/H	AI/H	H	H	H	H	NI	H	H	H	H/MI	AI	NI	H	
29	68	H	H	H	NI	AI	H	MI	H	MI	H	H	NI	H	MI	H	
30	63	H	H	H	AI/H	H	AI/H	H	NI	NI	H	H	AI	AI	H	AI	
31	54	NI	AI/H	H	H	H	H	H	H	H	H/MI	H	H	MI	H	H	
32	59	H	AI	NI	NI	H	H	H	NI	H	H	AI	AI	H	H	H	
33	65	H	H	H	H	H	NI	H	AI	AI	AI	H	H	H	H/MI	H	
34	52	AI	H	AI	AI	AI	AI	H	H	H	H	AI	H	H	H	AI	
35	47	H	AI	H	H	H	H	AI	NI	H	H	H/AI	H	H	H	H	
36	58	NI	H	NI	H	NI	H	H	H	H	NI	H	H	H	H	H	
37	53	AI	H	NI	H	AI	H	AI	AI	H	H	H	H	H	H	H	
AI (%)		29.6	28.6	22.2	18.2	32.3	29.0	10.0	25.0	15.2	15.6	11.1	5.9	6.1	3.1	11.4	
MI (%)		0.0	0.0	0.0	8.0	4.0	8.0	4.0	4.0	8.0	8.0	0.0	4.0	8.0	8.0	4.0	

NI, non-informative; H, heterozygote; AI, allelic imbalance; MI, microsatellite instability; /, alterations after radiotherapy.

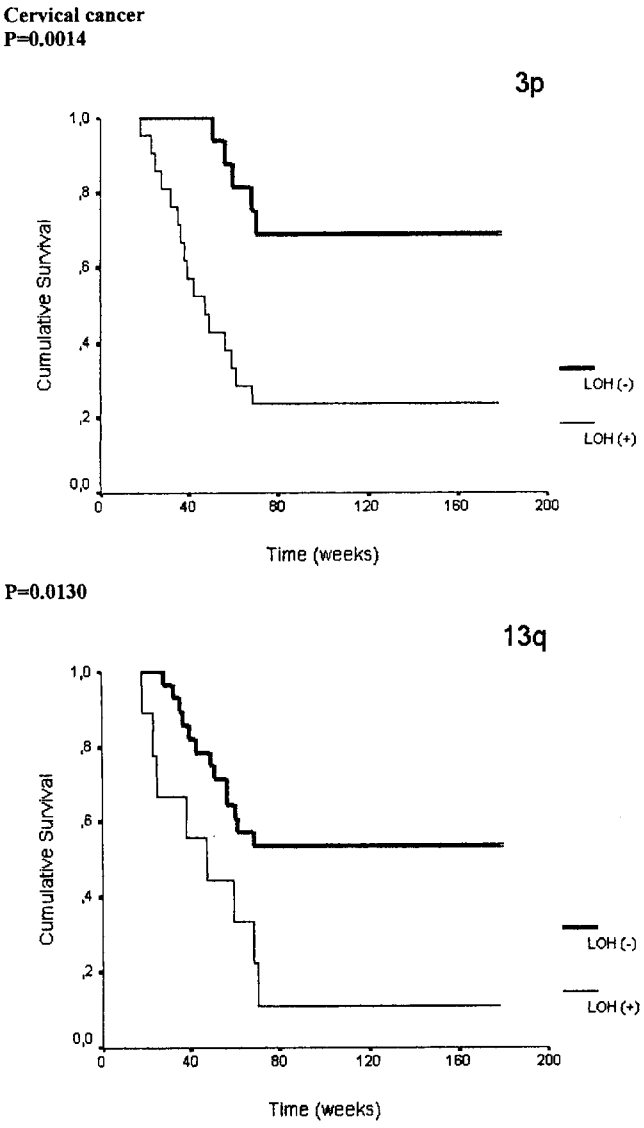

Figure 2. Kaplan-Meier survival curves for cervical cancer patients after radiotherapy with AI on 3p (upper panel) or 13q (lower panel) during follow-up.

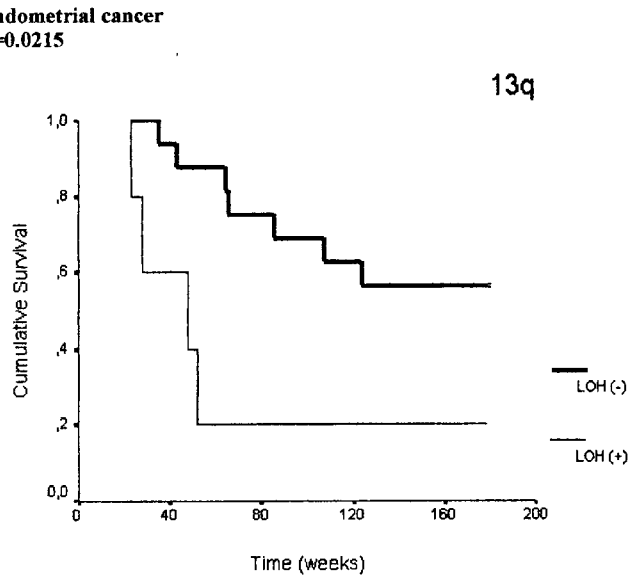

Figure 3. Kaplan-Meier survival curves for endometrial cancer patients after radiotherapy with AI on 13q during follow-up.

characterized as replication error-positive (RER$^+$) phenotype (15). D2S113, D9S161, D9S270, D13S290, D13S289, D17S579 and D17S855 were most frequently affected in as high as 8.0% of cases.

We also assayed 37 cervical cancer biopsies from the same cohort 3 months after the termination of radiotherapy. At least one genetic alteration of the initial tumor genotype was observed after radiation in 86.5% of cervical cancer cases. Reversion to the original normal genotype was also detected in 40.5% and novel genetic alterations in 75.7% of patients. Altered genotypes and cumulative results are shown in Tables III and V. The maximum observed number of such reversions in a single case was 5, seen in 2 patients who at present are alive and well, while 6 novel genetic alterations were seen in a patient who died of cancer.

At present, 16 patients (43.2%) have no evidence of disease (NED), 5 (13.5%) are alive with cancer (AWD) and 16 (43.2%) have died from recurrent disease (CD, cancer-caused death). Reversions were found to be more frequent in the NED group, 68.8%, 11 out of 16 patients, than in the AWD/CD group, 19.0%, 4 out of 21 (p=0.0028, Fisher's exact test). Novel genetic alterations were found in 62.5% of the NED and 85.7% of the AWD/CD group members but this difference was not statistically significant.

Survival curves by the Kaplan-Meier method (Fig. 2) showed a worse prognosis for cervical cancer patients whose tumors harbored AI before radiotherapy on 3p (p=0.0014, log-rank test) and 13q (p=0.0130, log-rank test). The cases of other chromosomal regions and the presence of AI or MI were examined by the log-rank test, but they did not show any statistical significance.

Endometrial cancer. Microsatellite DNA analysis was performed for 21 endometrial cancers versus venous blood DNA pairs before the initiation of radiotherapy with 31 markers. Seventeen out of 21 specimens (81.0%) exhibited AI in at least one marker, which most commonly affected the chromosomal regions 17q (52.4%) and 3p (42.9%), mean FRL values were 0.26 and 0.28 respectively. The microsatellite markers D3S1561 (33.3%), THRA1 (31.6%), D3S1260 (31.3%), D17S515 (30.0%), D1S116 (28.6%), D3S1611 (28.6%) and D17S855 (27.8%) exhibited most frequent AI (Table IV). The mean FAL value was 0.15 and the highest was 0.27 in a stage IV case, aged 72 years, who is alive with cancer. Cumulative data on endometrial cancer are presented in Table VI.

Four out of 21 patients (19.0%) exhibited MI in at least one marker before radiotherapy but none of these fulfilled the criterion of the MI co-finding on at least 5 markers to be characterized as replication error-positive (RER$^+$) (15). The microsatellite markers CLN1, MYCL1, D2S177, D2S113, D3S1611, D13S289 and THRA1 showed MI.

Table IV. Individual allelotypes for the endometrial cancer cases before and after radiotherapy.

A

Pt. No.	Age	Microsatellite DNA markers															
		D1S116	CLN1	MYCL1	D2S123	D2S177	D2S147	D2S2182	D2S288	D2S2291	D2S113	D2S138	D2S164	D2S105	D2S311	D3S1612	D3S1478
1	62	H	H	H	H	H	H	H	NI	H	H	H	H	H	H	AI/H	H
2	63	H	H	H	H/MI	H	H	H	H	H/MI	NI	NI	H	H	H	NI	H
3	58	AI/H	AI/H	H	NI	NI	H	H/MI	H	H	H	NI	H	H	H	H	H
4	72	NI	H	AI	H	H	H	H	NI	H	H	H	H	H	H	AI/H	AI/H
5	44	H	H	H	H	H	NI	H	H	H	NI	AI	H	H/MI	H	H	H
6	65	NI	H	H	H	NI	H	H	H	H	H	H	H	H	H	NI	AI
7	76	H/MI	NI	NI	H	H	H	NI	H	H/MI	H	H	H	H	H	H	H
8	39	AI	AI	H	NI	H	H	H	H/MI	H	H	NI	H	H	H	H	H
9	58	H	MI	MI	H	H	H	H	NI	H	H	H	H	H	AI	H	AI
10	55	AI	AI	NI	H	H/MI	H	NI	H	H	H	NI	H	H/MI	H	H	H
11	57	H	H	H	H	H	H	H	H	H	H	H	H	H	H	NI	H
12	47	NI	H	H	H	H	NI	H	H	H	NI	H	NI	NI	NI	H	H
13	66	N/MI	H	H	H	MI	H	H	NI	AI	MI	H	H	NI	H	H	H/AI
14	64	H	H	NI	H	NI	H	NI	NI	H	H	NI	H	NI	H	H	H
15	58	NI	H	AI	H	H/MI	H	NI	H	NI	H	H	H	H	H	AI	H
16	77	H	H	H	NI	H	NI	H	H	NI	NI	H	H	H	NI	NI	H
17	56	H	H	H	H	H	NI	H	NI	H	H	H	H	NI	H	H	H
18	44	NI	NI	H	AI	H	H	H	H	H/AI	H	H	NI	NI	H	AI	AI
19	53	H	H	H	H	H	H	H	NI	H	NI	NI	H	H	NI	H	H
20	49	AI	H	AI	NI	H	H	H	H	H	H	H	H	NI	H	H	AI
21	57	NI	H	NI	H/MI	H	H	H	H	NI	H	AI	NI	NI	AI	NI	H
AI (%)		28.6	16.7	18.8	5.9	0.0	0.0	0.0	0.0	5.6	0.0	13.3	0.0	0.0	11.1	25.0	23.8
MI (%)		0.0	4.0	4.0	0.0	4.0	0.0	0.0	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0	0.0

B

Pt. No.	Age	Microsatellite DNA markers														
		D3S1611	D3S1260	D3S1561	D9S161	IFNA	D9S270	D9S112	D13S22C	D13S290	D13S285	TP53	THRA1	D17S579	D17S855	D17S515
1	62	AI/H	AI/H	NI	H	NI	NI	H/MI	H	H	H	H	NI	H	H	AI
2	63	H	NI	H	H	H	H	H	AI	H	MI	AI	AI	NI	AI	H
3	58	H	H/MI	H	H	H	H	AI/H	H	H	H	H	H	H	H	AI
4	72	H	NI	AI/H	NI	AI	H	H	NI	H	H	H	AI	AI	H	H
5	44	AI	H	NI	H	H	H	H	H	AI/H	AI/H	H	AI	H	AI	AI
6	65	NI	AI	AI	AI/H	H	AI/H	H	NI	H	H	AI/H	H	H/MI	H	H
7	76	H	NI	H	H	NI	H	H	H	H	H	H	H	H	H	H
8	39	H	H	H	H	AI	NI	H	AI	AI	H	H	H	AI	H	AI
9	58	NI	H	AI	AI	AI	H/MI	H	H	H	H/MI	H	MI	H	AI	H
10	55	H	H	H	H	H	H	H	AI	H	AI	H	H	H	AI	AI
11	57	H	H	H	NI	H	H	H	H	NI	H	H	H	NI	H	H
12	47	AI/H	AI/H	AI/H	H	H/AI	NI	H	H	H	H	H	H	H	H	H/MI
13	66	NI	H	H	H	H	H	NI	H	H	NI	H	AI	AI	H	H
14	64	H	H	NI	H	H	H	H	NI	H	H	H	H	H	NI	H
15	58	AI	NI	AI	NI	H	H/MI	H	H	H	H	H	H	H	H/MI	H
16	77	H	H	H	AI	NI	NI	H	H	H	NI	AI	AI	H/AI	H	NI
17	56	NI	H	NI	H	NI	H	H/MI	NI	NI	H	H	AI	H	AI	AI
18	44	H	AI	H	H	NI	NI	H	H	NI	AI	H	H	H	NI	H
19	53	NI	H	NI	NI	H	H	H	NI	H	NI	H	H	H	H	H
20	49	NI	AI	NI	AI	H	AI	H	H	NI	H	H	H	H	NI	H
21	57	MI	NI	H/MI	H	H	H	NI	H	H/MI	H	AI	H/MI	NI	H	H
AI (%)		28.6	31.3	33.3	23.5	18.8	12.5	5.3	18.8	11.8	17.6	19.0	31.6	16.7	27.8	30.0
MI (%)		4.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0	4.0	0.0	0.0	0.0

NI, non-informative; H, heterozygote; AI, allelic imbalance; MI, microsatellite instability; /, alterations after radiotherapy.

Three months after the termination of radiation treatment, biopsy samples were obtained from the same area of the above endometrial tumors and examined for genetic alterations. The initial tumor genotype was found to be altered in 81.0% (Tables IV and VI). As observed in cervical cancer, endometrial cancers after radiotherapy also carried novel genetic alterations (76.2%), but lower reversions (28.6%).

At present, 7 patients (33.3%) have no evidence of disease (NED), 5 (23.8%) are alive with cancer (AWD) and 9 (42.9%) have died of recurrent disease (CD, cancer-caused death). Reversions were 10-fold more frequent in the NED group, 71.48%, 5 out of 7 patients, than in the AWD/CD group, 7.1%, 1 out of 14 (p=0.0054, Fisher's exact test).

Survival curves by the Kaplan-Meier method (Fig. 3) showed a worse prognosis for endometrial cancer patients whose tumors harbored AI before radiotherapy on 13q (p=0.0215, log-rank test). The cases of other chromosomal regions and the presence of AI or MI was examined by the log-rank test, but did not show any statistical significance.

Discussion

Cervical and endometrial cancers represent leading causes of female mortality from cancer worldwide (16). A proportion of these cancers either present at an advanced disease stage or at an advanced patient's age, for which the main treatment

Table V. Cumulative data on cervical cancer.

		Before radiotherapy											Genetic alterations after radiotherapy			
				Fractional regional loss												
Pt. no.	Age	MI	FAL	1p	2p	2q	3p	9p	9q	13q	17p	17q	Novel	Reversions	Total	Patient status
1	50	-	0.24	-	0.60	0.25	0.33	-	-	0.50	-	-	2	5	7	NED
2	74	-	0.29	1.00	-	0.40	0.75	-	-	-	-	-	2	2	4	AWD
3	55	-	0.12	-	-	0.25	-	1.00	-	-	-	-	3	-	3	AWD
4	68	2	0.15	-	-	0.20	0.33	-	-	1.00	-	-	3	-	3	CD
5	71	3	0.11	-	-	-	0.40	0.33	-	-	-	-	2	-	2	CD
6	52	-	-	-	-	-	-	-	-	-	-	-	1	-	1	NED
7	46	-	0.19	0.67	-	-	0.20	1.00	-	-	-	-	3	-	3	CD
8	45	4	0.24	-	0.20	0.20	0.75	-	-	-	-	0.33	4	-	4	CD
9	75	-	0.15	0.67	-	0.20	-	-	-	-	-	0.25	5	1	6	AWD
10	76	-	0.07	-	-	0.20	-	0.33	-	-	-	-	-	1	1	NED
11	51	1	-	-	-	-	-	-	-	-	-	-	-	-	-	NED
12	46	-	0.07	0.33	-	0.20	-	-	-	-	-	-	2	1	3	NED
13	58	1	0.26	-	-	0.25	0.40	0.67	1.00	0.33	-	-	3	-	3	CD
14	62	-	0.15	0.50	-	0.50	-	0.33	-	-	-	-	1	3	4	NED
15	67	-	0.11	0.67	-	-	-	0.33	-	-	-	-	2	-	2	CD
16	55	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NED
17	56	-	0.14	-	-	-	0.50	0.33	-	-	1.00	-	1	2	3	NED
18	64	-	0.22	-	-	0.40	0.25	0.33	-	1.00	-	-	3	-	3	CD
19	56	-	0.27	0.67	-	0.20	0.60	0.67	-	-	-	-	-	-	-	CD
20	50	-	0.27	-	-	0.67	0.50	-	-	1.00	-	-	1	-	1	CD
21	43	-	0.09	0.50	-	-	-	0.33	-	-	-	-	1	1	2	NED
22	62	1	0.25	-	-	0.25	0.50	0.50	-	1.00	-	-	3	-	3	CD
23	57	1	-	-	-	-	-	-	-	-	-	-	-	1	1	NED
24	51	-	0.41	-	0.60	0.33	0.67	-	-	0.67	-	0.33	6	-	6	CD
25	43	-	-	-	-	-	-	-	-	-	-	-	2	-	2	NED
26	56	-	0.15	-	-	0.50	0.50	-	-	-	-	-	1	2	3	NED
27	62	-	0.32	-	-	0.50	0.75	0.67	-	-	1.00	-	-	1	1	CD
28	50	-	0.32	1.00	-	0.25	0.75	-	-	-	-	0.33	1	5	6	NED
29	68	3	0.12	-	-	0.40	-	0.50	-	-	-	-	1	-	1	CD
30	63	-	0.27	-	0.50	-	-	0.67	-	-	-	0.75	-	2	2	NED
31	54	1	0.07	-	-	-	0.50	-	-	-	-	-	1	2	3	NED
32	59	3	0.25	1.00	-	-	0.67	-	-	-	1.00	0.25	1	2	3	CD
33	65	1	0.19	1.00	-	-	-	-	-	1.00	-	-	2	-	2	AWD
34	52	1	0.33	-	0.40	-	0.60	1.00	-	-	1.00	0.25	-	-	-	CD
35	47	2	0.15	-	-	0.67	0.25	-	1.00	-	-	-	2	-	2	AWD
36	58	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NED
37	53	-	0.21	-	-	-	0.50	0.33	1.00	0.33	-	-	2	-	2	CD
% of samples		35.1	83.8	29.7	13.5	54.1	56.8	45.9	27.0	24.3	13.5	18.9	75.7	40.5	86.5	
Mean		1.85	0.17	0.22	0.06	0.18	0.29	0.25	0.08	0.18	0.11	0.07	1.65	0.84	2.49	
SD		1.07	0.11	0.36	0.17	0.21	0.29	0.33	0.28	0.36	0.31	0.16	1.46	1.32	1.77	

FAL, fractional allelic loss; MI, microsatellite instability; NED, no evidence of disease; AWD, alive with disease; CD, cancer-caused death.

option has been radiotherapy. In order to identify new clinically relevant prognostic factors and to investigate the biological effects of radiation on these tumors, the present study was designed based on the high-throughput microsatellite DNA analysis.

We determined the genotypes of individual tumors compared to their correspondent normal DNA before radiotherapy on markers located at 1p32-p31, 2p22-p21, 2q21-q33, 3p24.2-p22, 9p21, 9q31-q34, 13q12.3, 17p13.1 and 17q21. This examination together with the follow-up study of patients,

Table VI. Cumulative data on endometrial cancer.

		Before radiotherapy											Genetic alterations after radiotherapy			
				Fractional regional loss												
Pt. no.	Age	MI	FAL	1p	2p	2q	3p	9p	9q	13q	17p	17q	Novel	Reversions	Total	Patient status
1	62	-	0.15	-	-	-	0.75	-	-	-	-	0.33	1	3	4	NED
2	63	1	0.16	-	-	-	-	-	-	0.50	1.00	0.67	2	-	2	CD
3	58	-	0.14	0.67	-	-	-	-	1.00	-	-	0.25	2	3	5	NED
4	72	-	0.27	0.50	-	-	0.75	0.50	-	-	-	0.50	-	3	3	AWD
5	44	-	0.25	-	-	0.25	0.25	-	-	0.67	-	0.75	1	2	3	NED
6	65	-	0.23	-	-	-	1.00	0.67	-	-	1.00	-	1	3	4	NED
7	76	-	-	-	-	-	-	-	-	-	-	-	2	-	2	CD
8	39	-	0.25	0.67	-	-	-	0.50	-	0.67	-	0.50	1	-	1	CD
9	58	3	0.23	-	-	0.20	0.50	0.67	-	-	-	0.33	2	-	2	AWD
10	55	-	0.21	1.00	-	-	-	-	-	0.67	-	0.50	2	-	2	CD
11	57	-	-	-	-	-	-	-	-	-	-	-	-	-	-	CD
12	47	-	0.13	-	-	-	0.60	-	-	-	-	-	2	3	5	NED
13	66	2	0.13	-	0.25	-	-	-	-	-	-	0.50	1	-	1	AWD
14	64	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NED
15	58	-	0.15	0.50	-	-	0.75	-	-	-	-	-	3	-	3	CD
16	77	-	0.14	-	-	-	-	1.00	-	-	1.00	0.33	1	-	1	CD
17	56	-	0.13	-	-	-	-	-	-	-	-	0.75	1	-	1	AWD
18	44	-	0.22	-	0.17	-	0.60	-	-	0.50	-	-	1	-	1	CD
19	53	-	-	-	-	-	-	-	-	-	-	-	-	-	-	NED
20	49	-	0.24	0.67	-	-	0.67	0.67	-	-	-	-	-	-	-	CD
21	57	1	0.14	-	-	0.67	-	-	-	-	1.00	-	4	-	4	AWD
% of samples		19.0	81.0	28.6	9.5	14.3	42.9	28.6	4.8	23.8	19.0	52.4	76.2	28.6	81.0	
Mean		1.75	0.15	0.19	0.02	0.05	0.28	0.19	0.05	0.14	0.19	0.26	1.29	0.81	2.10	
SD		0.96	0.09	0.32	0.06	0.16	0.36	0.32	0.22	0.27	0.40	0.28	1.06	1.33	1.64	

FAL, fractional allelic loss; MI, microsatellite instability; NED, no evidence of disease; AWD, alive with disease; CD, cancer-caused death.

revealed associations of AI on 3p24.2-p22 or 13q12.3 with poor response of cervical cancer and AI on 13q12.3 with poor response of endometrial cancer to radiotherapy. Since gain or loss of chromosomal material could affect expression of the genes located on this region (7), the results suggest the implication of the *hMLH1* (3p24.2-p22) and the *BRCA2* (13q12.3) genes, encoding for DNA repair enzymes of distinct pathways.

The *hMLH1* gene is part of the DNA mismatch repair system (MMR), which is involved in increasing the fidelity of replication by specific repair of DNA polymerase incorporation errors. MMR was discovered earlier in prokaryotes, but has been shown to be involved in cancer only within the past 8 years (15). In humans, two different heterodimeric complexes of MutS-related proteins (hMSH2-hMSH3 and hMSH2-hMSH6) and two different heterodimeric complexes of MutL-related proteins (hMLH1-hPMS2 and hMLH1-hPMS1) have been characterized as fundamental in both base and insertion/deletion mispairing (17,18). Mutations in *hMLH1* implicated in hereditary non-polyposis colorectal carcinoma (HNPCC), lacking its function produces the RER$^+$ phenotype (15). Tumors exhibiting RER$^+$ lacked detectable MMR activity in biochemical assays (19,20).

The *BRCA2* gene is part of another DNA repair system based on homologous recombination (HR). HR and non-homologous end joining (NHEJ) represent the two major mechanisms by which DNA double-strand breaks (DSBs) are repaired in mammalian cells (21). DSBs are responsible for radiation-induced cellular death (22), while they can also arise spontaneously. BRCA2 protein appears to be a positive regulator of HR interacting with Rad51, a central HR effector protein, and BRCA1, another regulator of HR (23). Mutations in *BRCA2* gene predispose carriers to breast cancer (24), while allelic loss at this locus was determined in 34% of sporadic breast tumors (25).

Thus, our data suggest that the function of HR is associated with cervical and endometrial tumor response to radiotherapy, while MMR deficiencies are important for the recovery of women from cervical cancer under the same treatment. No other genomic alteration, AI or MI, on any of the other chromosomal regions showed statistically significant correlation with clinical outcome of patients. This result is in

contradiction with previously reported correlation between microsatellite alterations on 17p13 and cervical cancer response to radiotherapy (9), probably because of the use of different microsatellite DNA markers. The incidence (13.5%) of such alterations in our study was determined using the TP53 marker.

This is the first report describing alterations after 3 months of radiation treatment in cervical or endometrial cancer, as a result of tumor cell progression or elimination and partly as novel radiation induced DNA damage incorporated in the genome of tumor or neighboring normal cells. Although these alterations occur randomly, reversions represent the recession of specific tumor clones. We found that in general NED cases exhibit higher frequencies of reversions than AWD and CD.

In conclusion, microsatellite DNA analysis before and after radiation could be used as a prognostic marker of cervical or endometrial tumor response to radiotherapy.

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