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Quantitative Analysis of *BRCA1*, *BRCA2* and *Hmsh2* mRNA Expression in Colorectal Lieberkühnien Adenocarcinomas and Matched Normal Mucosa: Relationship with Cellular Proliferation

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Abstract. *The human DNA mismatch repair gene hMSH2 is involved in the development of sporadic and hereditary nonpolyposis colorectal cancer. An increased risk of colorectal cancer has also been suggested in BRCA1 and BRCA2 mutation carriers. To address the relationship between the expression level of these genes and colorectal tumorigenesis, we studied BRCA1, BRCA2 and hMSH2 mRNA expression by real-time quantitative RT-PCR in 72 colorectal Lieberkühnien adenocarcinomas and matched normal mucosa. We investigated the relationship between mRNA levels and various clinicopathological parameters. The mean expression of BRCA1 3' and BRCA2 3' (mRNA pool), BRCA1 ex11 (with exon 11), BRCA2 ex12 (with exon 12) and hMSH2 mRNAs were increased in tumor samples. BRCA1 and BRCA2 mRNAs expressions were altered according to colon tumor site: BRCA1 3' and BRCA2 3' mRNAs levels were highest, respectively, in the right colon and left colon. No difference in hMSH2 mRNA levels was detected in relation to clinicopathological parameters. The mean SPF value was significantly higher in tumor than in non-tumor colonic tissue, and a high SPF value was correlated with high BRCA2 mRNA levels. BRCA2 3' mRNA levels tended to decrease as the Dukes' stage increased. In conclusion, the mechanisms of colorectal carcinogenesis seem to differ according to the right or left position of the tumor.*

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Colorectal cancer is an important public health issue because about 5% of persons will develop this disease. Sporadic colorectal cancer accounts for approximately 70% of all cancer. There are several molecular pathways to colorectal cancer, particularly the pathway involving abnormalities of DNA mismatch repair (MMR). Germ-line mutations in *MMR* genes have been reported in hereditary nonpolyposis colorectal cancer (HNPCC) (1, 2). Susceptibility to HNPCC is caused by mutations in one of the MMR genes (*hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, *hMSH6*). The majority of alterations have been found in *hMSH2* and *hMLH1*, which account for 45-65% of HNPCCs (3, 4). Mutations in other MMR genes appear to be rare, absent and/or associated with atypical families (1-5%). Mutations of *hMSH2* and *hMLH1* genes are found in about 15% of sporadic colorectal carcinomas and are associated with microsatellite instability (5, 6). However, most sporadic colorectal carcinomas seem to be related to epigenetic rather than genetic changes (7). On the other hand, an increased risk of colorectal cancer has been found in *BRCA1* and *BRCA2* mutation carriers (8, 9). Germ-line mutations in the breast susceptibility genes *BRCA1* and *BRCA2* confer a high risk for developing breast and ovarian cancer (10, 11). Women who develop two of these cancers may be expected to have a constitutionally increased risk of developing subsequent cancers to which mutations in these genes predispose. Cancers for which an increased relative risk has been found in *BRCA1* and *BRCA2* mutation carriers include colon, pancreatic, gall-bladder cancers and melanoma (12, 13). BRCA1 and BRCA2 regulate multiple nuclear processes including DNA repair, cell cycle checkpoints and transcription (14). These proteins have been shown to interact with DNA repair proteins. Wang *et al.* demonstrated an interaction between BRCA1 and hMSH2 *in vitro* and *in vivo* (15). Moreover, BRCA1 and

Table I. *Clinical and histological characteristics of patients.*

Variable		Number of cases	(%)
Sex	Male	38	(52.8)
	Female	34	(47.2)
Age (years)	< 50	3	(4.2)
	51 - 64	17	(23.6)
	65 - 80	40	(55.5)
	≥ 80	12	(16.7)
Anatomic Site	Right colon	20	(27.8)
	Left colon	52	(72.2)
Tumor Size (cm)	< 3	6	(8.3)
	3 - 4.9	33	(45.8)
	5 - 6.9	22	(30.6)
	≥ 7	10	(13.9)
	NA[a]	1	(1.4)
Dukes' Stage	A	3	(4.2)
	B	41	(56.9)
	C	24	(33.3)
	D	4	(5.6)
pN[b]	-	45	(62.5)
	+	26	(36.1)
	NA	1	(1.4)

[a]NA: not available
[b]pN: histological lymph node involvement

BRCA2 are part of a large multisubunit protein complex of tumor suppressors, DNA damage sensors and signal transducers. This complex, named BASC for BRCA1-Associated Genome Surveillance Complex, is composed of DNA repair proteins: hMSH2, ATM, BLM, hMSH6, hMLH1, RAD51, the RAD50-MRE11-NBS1 complex and the RFC1-RFC2-RFC4 complex. BASC may serve as a sensor of abnormal DNA structures and/or as a regulator of post-replication repair (16). The BRCA1 and BRCA2 splicing variants may have possible cellular roles (35), which could explain why, despite its vital cellular functions, mutations of this gene are associated with tissue-specific tumor formation in the breast and the ovary. The major splicing variants are those lacking exon 11 (*Δ11BRCA1*) for *BRCA1* and exon 12 (*Δ12BRCA2*) for *BRCA2*. With regard to the relationship between BRCA1, BRCA2 and hMSH2 functions and their involvement in tumorogenesis, we studied mRNA expression of these genes in 72 Lieberkühnien adenocarcinomas and matched normal mucosa. By real-time quantitative RT-PCR, we quantified *hMSH2*, *BRCA1 3'* and *BRCA2 3'* (mRNA pool), *BRCA1 ex11* (transcripts containing exon 11) and *BRCA2 ex12* (transcripts containing exon 12) mRNAs. These mRNA levels were compared to various clinicopathological parameters in order to clarify their roles in colorectal carcinogenesis.

Materials and Methods

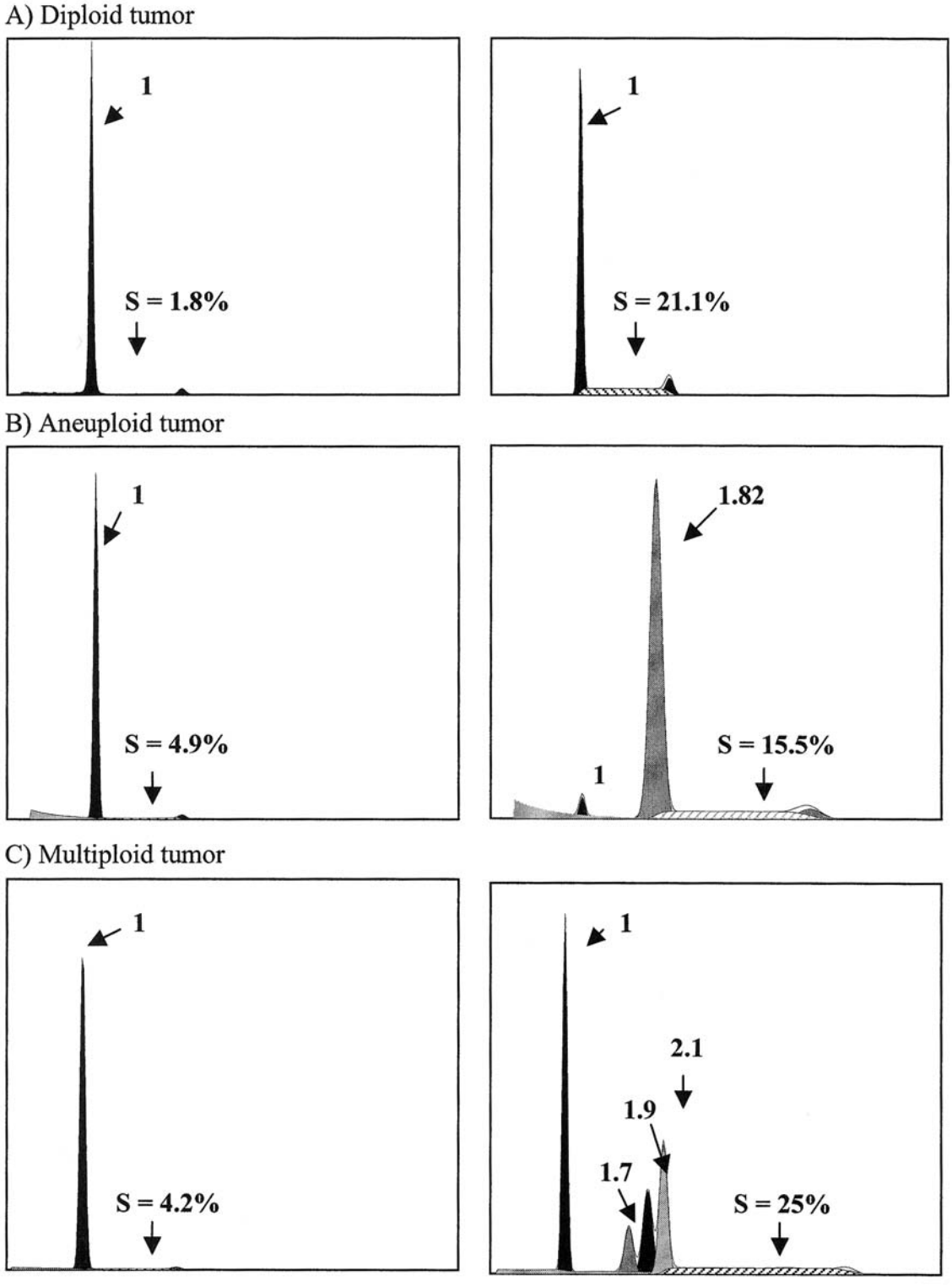
Patients and samples. Seventy-two pairs of histologically verified sporadic colorectal cancer (with no familial or inherited predisposition) and adjacent normal colorectal mucosa were obtained at the time of surgery and collected as frozen samples in Centre Paul Papin, Angers (France). None of the patients received radiotherapy or chemotherapy before surgery. There were 38 men and 34 women, with a mean age of 69.8 years (range 32 to 90 years). All samples were staged according to Dukes' classification modified by Gunderson and Sosin (17). All tumors were Lieberkühnien adenocarcinomas and ranged from poorly- to well-differentiated. The clinical and histological characteristics of the patients are reported in Table I.

Flow cytometric (FCM) DNA-ploidy analysis and S-phase (SPF) measurement. For each patient, one tumor fragment was compared with a matched non-tumor colonic tissue. DNA analyses were performed in the same experiment by preparing a mechanical dissociation of the thawed samples. Vindelov's protocol was used to stain nuclear DNA (18). FCM analysis was performed with a FACScan flow cytometer (Becton Dickinson, CA, USA) equipped with Modfit 5.2 software (Verity Software House, Topsham, ME, USA). Every cell suspension was analyzed in the same conditions in order to locate the DNA-diploid peak on DNA histograms according to an external standardization procedure using normal colonic cells positioned at the fifth part of the red fluorescence scale.

A tumor sample was DNA-diploid (D) when it provided a unimodal histogram with a G_0G_1 peak in the same channel as normal cells (+/-1%) (Figure 1-A). A sample was classified as aneuploid when a second peak exceeded 5% of total cells, and tetraploid when this proportion exceeded 10% with a corresponding DNA index between 1.9 and 2.1 (Figure 1-B). For some near-diploid cases, the tumor cell suspension was mixed with non-tumor cells before staining in order to ensure the position of the diploid peak. Finally, tumors were characterized as multiploid with different aneuploid populations (Figure 1-C).

The S-phase fraction was calculated when the background was lower than 20% of total acquired events and the coefficient of variation of the G_0G_1 peak was lower than 5%. When a unimodal histogram was obtained, the diploid option of the software was used. In case of abnormal DNA content, only the aneuploid SPF was taken into account, provided that the aneuploid fraction exceeded 30% of cells. In every case, the rectangular option was chosen for SPF calculation and the background subtraction option was always used.

Real-time RT-PCR. Colorectal tumors and normal mucosa were ground thoroughly with a french press and the powder was dispersed in a crucible with the appropriate amount of RTL™ buffer supplemented with β-mercaptoethanol and GITC (guanidinium isothiocyanate buffer), from the RNEasy kit as recommended by the manufacturer (Qiagen, Courtabœuf, France). The solution was homogenized by passing the lysate at least 10 times through a 30-gauge needle fitted to a RNase-free syringe. One μg of RNA was taken for reverse transcription with the First Strand cDNA Synthesis kit (Amersham Pharmacia Biotech, Uppsala, Sweden). For *BRCA1*,



Left column: DNA histograms obtained in normal colonic tissues
Right column: DNA histograms obtained in tumor samples
DNA index are shown above each G_0G_1 peak.

Figure 1. *DNA histograms obtained in three different colonic carcinomas.*

BRCA2 and *hMSH2* mRNA analysis, probes and primers (Table II) were designed using the Primer Express software (Applied Biosystems, ZA Courtabœuf, Villebon/Yvette, France) so that they overlapped splice junctions (exons 23-24 for *BRCA1 3'* probe, exons 11-12 for *BRCA1 ex11* probe, exons 26-27 for *BRCA2 3'* probe, exons 12-13 for *BRCA2 ex12* probe, exons 9-10 for *hMSH2* probe), to distinguish PCR products generated from genomic *versus* cDNA templates. The sequence of forward primers, TaqMan® probes and reverse primers were, respectively, for *BRCA1-ex 3'*: 5'-^{5566}CAGAGGACAATGGCTTCCATG^{5586}-3', 5'-^{5588}AATTGGGC AGATGTGTGAGGCACCTG^{5613}-3', 5'-^{5646}CTACACTGTCCAA CACCCACTCTC^{5623}-3'; for *BRCA1-ex 11* amplification: 5'-^{4157}AAGAGGAACGGGCTTGGAA^{4175}-3', 5'-^{4177}AAAATAATC AAGAAGAGCAAAGCATGGATTCAAACTTA^{4214}-3', 5'^{4236}CACACCCAGATGCTGCTTCA^{4217}-3'; for *BRCA2-ex 3'* amplification: 5'-^{9794}CCAAGTGGTCCACCCCAAC^{9812}-3', 5'-^{9818} ACTGTACTTCAGGGCCGTACACTGCTCAAA^{9847}-3', 5'-^{9895}CA CAATTAGGAGAAGACATCAGAAGC^{9870}-3'; for *BRCA2-ex 12* amplification: 5'-^{7120}GAAAATCAAGAAAAATCCTTAAAGG CT^{7147}-3', 5'-^{7153}AGCACTCCAGATGGCACAATAAAAGATC GAAG^{7184}-3', 5'-^{7220}GTAATCGGC TCTAAAGAAACATGA TG^{7195}-3'; for *hMSH2* amplification: 5'-^{1451}AATAATGAATGA CTTGGAAAAGAAGATG^{1479}-3', 5'-^{1498}CAGCCAGAGATCTT GGCTTGGACCCT^{1524}-3', 5'-^{1552}CACTGGAATCCAGTTTAAT CTGTTTG^{1527}-3'. All doubly-labelled probes, 18S rRNA probe, primers plus TaqMan universal PCR Master Mix were obtained from Applied Biosystems. Real-time PCR reactions were conducted in 96-well plates on cDNA equivalent to 10 ng of total RNA. A typical 25 μl reaction sample contained 12.5 μl TaqMan universal PCR Master Mix [containing 1X TaqMan buffer, 200 μM dATP, dCTP, dGTP, and 400 μM dUTP, 5 mM $MgCl_2$, 1.25 units of AmpliTaqGold, 0.5 unit of Amperase uracil-N-glycolsylase (UNG)], 200 nM of *BRCA1*, *BRCA2* or *hMSH2* primers and 50 nM of 18S rRNA primers, 200 nM of *BRCA1*, *BRCA2* or *hMSH2* TaqMan® probes and 50 nM of 18S rRNA TaqMan® probe. Thermal cycling conditions were 2 min at 50˚C and 10 min at 95˚C, followed by 40 cycles at 95˚C for 15 sec and 60˚C for 1 min. Data were collected using the ABI PRISM 7700 SDS analytical thermal cycler (Applied Biosystems). Each sample was tested in triplicate.

Assuming the same amplification efficiency for target and reference genes, quantification was performed using the comparative *Ct* method (19), which consists of the normalization of the number of target gene copies to an endogenous reference gene (*i.e.* 18S rRNA), designated as the calibrator. The parameter *Ct* is defined as the cycle number at which the signal is detected. The amount of target, normalized to the 18S rRNA endogenous reference, is given by the formula: $2^{-\Delta\Delta CT}$, where $\Delta\Delta C_T = \Delta C_{T\ tumor} - \Delta C_{T\ mucosa}$ and $\Delta C_T = C_{T\ target} - C_{T\ 18S\ rRNA}$. The level of mRNA expression in colorectal tumor was normalized to the non-tumor colonic tissue.

To ensure the reproducibility of the determination of mRNA in each sample, two independent RNA extractions were done. With one RNA extraction, two independent reverse transcriptions were done. With the second RNA extraction, only one reverse transcription was performed. For the three reverse transcriptions, one determination was done with the ΔC_T formula. Each determination was done in triplicate and expressed as mean ± SD (20).

Statistical analysis. Mean values of two series were compared using the Student's *t*-test and Anova test when more than two series were concerned. Two-sided *p* values of <0.05 were considered significant. The χ_2-test was used to determine the significance of the relationship between DNA ploidy and other variables. The levels of mRNA expression were categorized in two groups (1: low values and 2: high values) according to their respective 75th percentile values as follows: 6.3 for *BRCA1 3'*, 5.4 for *BRCA1 ex11*, 14.0 for *BRCA2 3'*, 11.4 for *BRCA2 ex12* and 4.9 for *hMSH2*.

Table II. *Mean values and 75th percentiles of mRNA gene expression.*

	Number of cases	Mean value	SD	75th percentile
BRCA1 3'	72	4.9	4.8	6.3
BRCA1 ex11	72	3.5	3.3	5.4
BRCA2 3'	71	12.0	13.6	14.0
BRCA2 ex12	71	9.7	11.1	11.4
HMSH2	72	3.5	11.4	4.9

The mean value (± SD) corresponds to mRNA expression levels in tumor samples normalized to the normal colonic tissue. Each measurement was performed on two extractions and three RT.

Results

In the clinical and histological characteristics shown in Table I, 20 tumors were localized in the right colon (ascending and transverse) and 52 in the left colon (descending, rectosigmoid and rectum). The mean tumor size was 4.5 cm (range 1.5-9 cm). Most of the tumors were classified as Dukes' B stage (56.9%) and 4 patients had metastases at diagnosis. Twenty-six patients had histological lymph node involvement.

Flow cytometric DNA analysis

DNA ploidy. The DNA content was always normal in healthy colonic mucosa, while 47 tumors (65.3%) were classified as aneuploid (Figure 1-B and 1-C). Among these, the DNA index showed very scattered values (range 0.9-3.1) and 6 tumors were multiploid. The frequency of aneuploidy did not correlate significantly with anatomic site. DNA ploidy did not correlate with Dukes' stage, the frequency of aneuploidy being 65.9% in Dukes' B and 70.8% in Dukes' C.

S-phase fraction (SPF). Owing to good quality DNA histograms (debris mean 7.7% and CV mean 2.9%), SPF could be calculated in 71 out of 72 tumors. The mean SPF value was significantly higher in tumor than in non-tumor colonic tissue (15.1% and 3.1%, respectively, $p<10^{-6}$). The diploid SPF mean value was not significantly lower than the aneuploid SPF mean value (13.5% and 16.7%, respectively, $p=0.078$). SPF did not correlate with tumor site or Dukes' stage (SPF mean values were 15.9%, 14.9% and 13.9% in Dukes' B, C and D, respectively).

Table III. *Mean expression (± SD) of mRNA according to tumor site.*

	Right colon 20*		Left colon 52	
	Mean	S.D.	Mean	S.D.
BRCA1 3'	6.89[a]	5.38	4.09	4.36
BRCA1 ex11	3.61	3.25	3.49	3.30
BRCA2 3'	7.01	5.14	13.90[b]	15.30
BRCA2 ex12	6.52	5.61	10.94	12.41
HMSH2	3.53	2.33	3.47	2.59

The level of mRNA expression in colorectal tumor was normalized to the non-tumor colonic tissue. Each measurement was performed on two extractions and three RT. Statistical analysis was performed using Student's *t*-test.
[a]mean expression of *BRCA1 3'* mRNA is higher in right colon compared with left colon ($p=0.038$)
[b]mean expression of of *BRCA2 3'* mRNA is higher in left colon than in right colon ($p=0.005$)
*number of tumors

Table IV. *Mean expression of mRNA BRCA2 3' according to Dukes' stage.*

	Dukes' stage	A 3*	B 41	C 23	D 4
BRCA2 3'	Mean expression	6.4	15	8.7	3.6
	SD[a]	1.8	16.6	6.1	1.4
	P(Anova test)			0.028	

The level of mRNA expression in colorectal tumor was normalized to the non-tumor colonic tissue. Each measurement was performed on two extractions and three RT. Statistical analysis was performed using Anova test.
* number of tumors
[a]SD: standard deviation

Table V. *Mean SPF values according to gene mRNA expression.*

mRNA expression	Mean	SPF (%)	*P* value
BRCA1 3'	< 6.3	16.1	
	> 6.3	14.0	NS[a]
BRCA1 ex11	< 5.4	15.6	
	> 5.4	15.4	NS
BRCA2 3'	< 14.0	14.1	
	> 14.0	19.9	0.0013
BRCA2 ex12	< 11.4	14.7	
	> 11.4	17.1	NS
hMSH2	< 4.9	15.5	
	> 4.9	15.5	NS

Gene mRNA expressions are expressed according to 75th percentile (52 cases are classified in the "low" group and 20 cases are classified in the "high" group). Statistical analysis was performed using Student's *t*-test.
[a]NS: no significant value.

Quantitative analysis of BRCA1, BRCA2 and hMSH2 mRNA expression. The mean values of mRNA levels for each gene are reported in Table II. The distribution of these values indicated a few tumors with high mRNA expression. For each gene, tumors were dichotomized, using the 75th percentile as a cut-off point for high and low expression levels (Table II). Thus, a quartile of tumors with high mRNA gene expression was discriminated.

Correlations with histological characteristics. BRCA1 ex11, *BRCA ex12* and *hMSH2* mRNA levels were not altered according to tumor site. *BRCA1 3'* mRNA levels were highest in the right colon ($p=0.038$) and *BRCA2 3'* mRNA levels were highest in the left colon ($p=0.005$) (Table III). *BRCA2* expression showed a discrepancy with Dukes' stage, with *BRCA2 3'* mRNA levels decreasing as the histological stages progressed by Dukes' classification (Table IV).

Correlation with FCM results. BRCA1, *BRCA2* and *hMSH2* mRNA levels were statistically independent of ploidy. In contrast to the other mRNA, a significant correlation was observed between SPF and *BRCA2 3'* mRNA expression ($p=0.0013$). Moreover, the mean SPF value appeared to be higher in a quartile of tumors exhibiting *BRCA2 3'* mRNA levels higher than 14.0 (Table V). This result is more clearly observed in tumors of the left colon, which exhibit the highest mRNA expression values (Table III).

Discussion

In order to elucidate the potential roles of *hMSH2*, *BRCA1* and *BRCA2* genes in sporadic colorectal cancer, we quantified the mRNA levels of these genes and compared their expression levels with various clinicopathological tumor parameters. *BRCA1 3'*, *BRCA2 3'* and *hMSH2* probes were designed to quantify the expression of all *BRCA1*, *BRCA2* or *hMSH2* species together, because no alternative splicing of exon 23 (for *BRCA1*), exon 26 (for *BRCA2*) and exon 9 (for *hMSH2*) has been described. Statistical analysis showed that there was no correlation between *BRCA1*, *BRCA2* and *hMSH2* mRNA levels and patient age or sex, DNA-ploidy and tumor size. Previous studies have shown the involvement of *hMSH2* in the MMR system (21). Moreover, the majority of the alterations in HNPCC and sporadic colorectal carcinomas are down-regulation of *hMSH2* and *hMLH1* gene expression (1, 2). In our observations, the *hMSH2* mRNA level appeared to be up-regulated in colorectal Lieberkühnien adenocarcinomas. It has been shown that the steady-state levels of *hMSH2* protein are very low in lysates of resting cells, but that a

considerable increase in expression is observed when cells enter the cell cycle (22, 23). Marra *et al.* suggested that increased hMSH2 protein expression is required when DNA replication in S-phase is activated and followed by mitosis. These observations are consistent with our results, where the mean SPF value was significantly higher in tumors than in normal colonic tissues. Thus, the up-regulation of *hMSH2* mRNA expression in colorectal adenocarcinomas seems to be induced by the high proliferation and, consequently, the high SPF values in tumors.

Epidemiological studies hint at a *BRCA1* and *BRCA2* role in hereditary colorectal cancer (12, 13). The *BRCA1* and *BRCA2* genes account for about 5-10% of breast cancer. *BRCA1* and *BRCA2* mutations appear to be responsible for up to 80% of hereditary breast cancer (10, 11), but not for sporadic breast cancer where *BRCA1* and *BRCA2* expression is down-regulated mainly by epigenetic changes (24-26). We found an up-regulation of *BRCA1* and *BRCA2* mRNA levels in sporadic colorectal tumor compared with normal colonic tissue. *BRCA1* and *BRCA2* mRNA expression is reported to be up-regulated *in vitro* in certain malignant human ovarian, prostate and breast cancer cell lines (27) and *in vivo* in no *BRCA1*-associated sporadic breast cancer (28, 29). Moreover, BRCA1 and BRCA2 expression increases during late G_1- and S- phase of the cell cycle (30, 31). Our present observations, that the mean *BRCA1* and *BRCA2* mRNA levels and SPF values were significantly higher in colorectal adenocarcinomas than matched normal mucosas, seem to be consistent with this report. One possible explanation for these results is that *BRCA1* and *BRCA2* mRNA expression is induced by proliferation, as demonstrated in previous studies (32, 33). The BRCA1 and BRCA2 proteins are believed to exert growth-inhibitory action. Thus, an increase of their mRNA level in sporadic colorectal cancer may represent a negative feedback mechanism for curbing proliferation in fast-growing cells.

Among the clinicopathological parameters, *BRCA1* and *BRCA2* mRNA expressions differed with tumor site. Thus, *BRCA1 3'* mRNA level was higher in the right colon and *BRCA2 3'* mRNA level was higher in the left colon. Previously, the same relationship was established with p53 expression, which showed more p53 expression in distal than proximal neoplasms (34). These data support the hypothesis that mechanisms of colorectal carcinogenesis may differ in the right and left colon. On the other hand, only *BRCA2* expression varied with SPF and Dukes' stage, as *BRCA2* 3' mRNA level tended to decrease as the histological stage increased in Dukes' classification (Table V). Our results indicate that the cell cycle of tumor cells is disturbed through the reduction in *BRCA2 3'* mRNA expression, leading to uncontrolled cell proliferation. The tumor cells may acquire more rapid growth activity and more malignant potential as the *BRCA2 3'* mRNA expression level decreases.

The number of known *BRCA1* and *BRCA2* mRNA variants is relatively high, with remarkably different expression patterns (10, 35-37). Although their possible functions are still unclear, the splicing variants may have possible cellular roles (38). Splicing variants of these genes could explain the current paradox that, despite its vital cellular functions, mutations of this gene are associated with tissue-specific tumor formation in the breast and the ovary. The variants that would be expected to differ greatest at the functional level from the full-length species are those lacking exon 11 (*Δ11BRCA1*) for *BRCA1* and exon 12 (*Δ12BRCA2*) for *BRCA2*, as a result of their remarkable size difference and the absence of many functional domains involved in protein-protein interactions (36, 37, 39). In order to define a possible implication of splice variants in colorectal cancer, we quantified *BRCA1 3'* and *BRCA2 3'* (mRNA pool), *BRCA1 ex11* (transcripts with exon 11) and *BRCA2 ex12* (transcripts with exon 12). All the mean values of *BRCA1* and *BRCA2* mRNA expression were increased in colorectal tumor in comparison with normal tissue. Moreover, the contribution of *BRCA1* and *BRCA2* splice variants, in particular *Δ11BRCA1* and *Δ12BRCA2* mRNA, appears weak in the up-regulation of *BRCA1* and *BRCA2* mRNA levels. These results suggest than *BRCA1* and *BRCA2* mRNA variants seem not to be implicated in the carcinogenesis of colorectal Lieberkühnien adenocarcinomas.

In conclusion, pathologically decreased *hMSH2*, *BRCA1* and *BRCA2* mRNA levels do not seem to account for all tumors in this population-based series of colorectal Lieberkühnien adenocarcinomas. On the contrary, our data suggest that these mRNA are up-regulated in response to proliferation. Moreover, mechanisms of colorectal carcinogenesis seem to differ according to tumor site and only *BRCA2* mRNA seems to be up-regulated with proliferation and genomic instability in high Dukes' stage of tumors. In order to better understand colorectal tumorogenesis, the genetic mechanisms inducing alterations of *hMSH2*, *BRCA1* and *BRCA2* mRNA expression need to be studied.

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