Mismatch DNA Repair hMSH2, hMLH1, hMSH6 and hPMS2 mRNA Expression Profiles in Colorectal Carcinomas

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Abstract

Background: Mismatch repair (MMR) deficiency has been related with HNPCCs. So far, there is limited information on MMR mRNA profiles in sporadic colorectal carcinomas (CRCs). We previously showed that distinct MMR mRNA phenotypes were related to tumor stage and survival of patients with lung cancer or urinary bladder carcinomas.

Aim: The aim of this study was to quantify hMSH2, hMLH1, hMSH6 and hPMS2 mRNA levels, in CRCs and their adjacent normal tissues (ANTs), using accurate methodology, and to correlate MMR mRNA profiles with patient or tumor characteristics.

Materials and methods: We analyzed 31 fresh frozen tissue specimens of paired CRCs with their ANT. We evaluated MMR mRNA profiles by a Q-real time PCR, using hPBGD gene as reference control and creating a standard curve. The MMR mRNA levels were assigned as ratios MMR/hPBGD mRNAs. Relative expression of each MMR gene was given as ratios of CRCs/ANTs mRNA levels.

Results: All CRCs and their ANT expressed low hPMS2 mRNA levels while a significant proportion of CRCs (73%) and their ANT (82%) presented low hMSH2 mRNA levels. Analysis of relative expression patterns showed that hMSH6 and hMLH1 exhibited the highest percentages of reduction (53% and 45.5%, respectively). We found a correlation of transcriptional levels between hMSH2 and hMLH1, the crucial components of MMR mechanism and between their counterparts, hMSH6 and hPMS2, in CRCs of early stages, related to gender. On the contrary, CRCs of late stages revealed a correlation between reduced levels of hMSH2 and hMSH6, MutSa components, unrelated to gender but related to lymph node metastasis. Also, reduced hMSH2, hMSH6 and hMLH1 mRNA phenotypes correlated with advanced stage, and rectal localization.

Conclusion: In this study we demonstrated that MMR mRNA deficiency is a common event in sporadic CRCs. Specific profiles of MMR deficiency may be related to tumor progression, especially in male patients.

Keywords: Colorectal carcinomas; hMSH2; hMLH1; hMSH6; hPMS2; mRNA; Real time PCR; MMR phenotypes

Introduction

Mismatch DNA repair (MMR) mechanism protect cells from replication errors and is important for genome stability [1-3]. Hereditary cancers, like Lynch syndrome, are linked with a deficiency of MMR mechanism [4-13]. Sporadic cancers have also been related with defective MMR mechanisms [14-24]. We have recently demonstrated that distinct MMR mRNA profiles are related to tumor stage and survival of patients with non-small cell lung carcinomas (NSCLCs) [16,17]. We have also showed dependence of hMSH2 and hMSH6 mRNA expression in urinary bladder carcinomas (UCCs) and revealed a correlation of hMSH6 reduction in UCCs (18). So far, there is a limited information on transcriptional levels of MMR genes in sporadic colorectal carcinomas (CRCs) [25,26].

In this study we quantified, with a precise Q-real time PCR method, the transcriptional levels of hMSH2, hMLH1, hMSH6 and hPMS2, MMR genes in CRCs and their paired adjacent normal tissues (ANTs) and we checked for correlations of MMR mRNA CRC profiles with tumor or patients’ characteristics.

Materials and Methods

Tissue collection

Fresh frozen colorectal tissue specimens consisting of paired tumor and their adjacent normal tissues (ANTs), were collected from 31 unselected patients, 17 male and 14 female with an age range 52-92 years (median 74 years), who underwent surgery at the University Hospital of Larissa, Thessaly, Greece. The specimens were immersed immediately after surgery in RNA stabilizer solution (RNAlater®, Life Technologies) and they were preserved in -80°C, deep freezer, till RNA isolation. The localization of the tumor was rectum (11), right colon (10 cases), sigmoid (6) and left colon (1) while in three cases the exact localization was not available (Table 1). The histological review showed that all colorectal tumors of our collection were adenocarcinomas consisting of I well differentiated (WD), 24 moderately-differentiated (MD), 3 moderately to poorly differentiated (MD-PD) and 3 poorly differentiated (PD) tumors. Lymph node metastasis, nerve and vascular invasion was observed in 11/31 (35.5%), 18/31 (58%) and 13/31 (42%) out of total 31 of CRC tumors, respectively (Table 1).

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compared with that of the corresponding ANT samples. This value is 

\[ r = \frac{\text{MMR gene expression of tumor samples}}{\text{MMR gene expression of ANT samples}} \]

for mRNA ratios <1 and 

\[ r = \frac{\text{MMR mRNA (tumor/control mRNAs)}}{\text{MMR mRNA (control mRNAs)}} \]

defined two 
copies, as previously described [16,27]. The mRNA 
quantitation of mRNAs was achieved creating a standard curve of serial 
dilutions of 
hPBGD by Q-RT-PCR. These data are summarized in 

Table 1: Quantitative mRNA expression of MMR DNA repair genes in colorectal adenocarcinomas and their adjacent normal tissues.

**Quantitative analysis of MMR mRNA expression**

We used RNAeasy kit (Qiagen®, USA) for total RNA isolation and 

Super Script First-Strand Synthesis System (Invitrogen®, Life 
Technologies, UK) for cDNA synthesis, according the manufacturer’s 

instructions. The qPCR analysis of hMSH2, hMSH6, hPMS2 and 
hPBGD-control mRNA was performed using specific primers previously 

published (16,18) and probes for hMSH2: 5’-6FAM-CATATAAGGCTTCTGGCG-BHQ1-3’, for hMSH6: 5’-6FAM-CAGGAAGCTTCTCAAGGCAGTCA-BHQ3-3’, for hPMS2: 5’-6FAM-AGCTGCTTTAACACAAGGGGATAGAA-BHQ1-3’ and for 

hPBGD: 5’-6FAM-CCTGTCGGCCTTCTCTGCTGCA-BHQ1-3’, designed for RotorGene 6.1 instrument (CORBETT Research, Australia) using Platinum® Quantitative PCR Super Mix-UQ (Invitrogen®, Life 

Technologies, UK) and annealing temperatures at 58°C for hMSH2 and 

hPBGD, at 54°C for hMSH6 and hPMS2. The qPCR analysis of hMLH1 was performed using Quantifast Probe Assay SP kit (Qiagen®, USA) by 

applying in Rotor Gene 6.1 instrument, according the instructions. The 

quantification of mRNAs was achieved creating a standard curve of 

serial dilutions of hPBGD copies, as previously described [16,27]. The mRNA 

expression of each MMR gene was expressed as a ratio of MMR mRNA to 

control hPBGD mRNAs (MMR/control mRNAs) and defined two 

major phenotypic groups, the reduced (r or p) for mRNA ratios <1 and 

the normal or elevated (R or P) for ratios ≥1, as previously described 

[16,18]. Additionally, the MMR gene expression of tumor samples was 

compared with that of the corresponding ANT samples. This value is 

indicated as relative mRNA expression of MMR genes between CRC tumors and ANTs (CRCs/ANT) of each patient (Table 1).

**Statistical analysis**

We used the paired Student’s t-test to compare ratios of hMSH2, 
hMLH1, hMSH6 and hPMS2 alterations between tumor and matched 

ANTS for different patient characteristics, including age, gender and clinical or histopathological parameters such as tumor location, grade, 

stage and lymph node metastasis, vascular invasion or perineural invasion. The correlation between the mRNA expression ratios of 

hMSH2, hMLH1, hMSH6 and hPMS2 in CRCs and their ANTs for 

different patient and tumor characteristics was examined by 

Pearson test. The \( \chi^2 \) test was also used to examine the distribution of MMR mRNA phenotypes in tumor and ANT specimens at different tumor 

histological grades or stages or lymph node or nerve or vessel filtration or tumor location (Tables 2-4).

**Results**

hMSH2 & hMLH1 & hMSH6 & hPMS2 mRNA quantification in CRCs and their ANTs

We evaluated hMSH2, hMLH1, hMSH6 and hPMS2 mRNA levels in 

primary CRCs and their corresponding ANTs relative to the hPBGD control gene by Q-RT-PCR. These data are summarized in 

Table 1 along with clinical and histopathological data.
All CRCs and their ANTs (100%) revealed low mRNA levels (MMR/control mRNA ratios <1) of hMSH6 and hPMS2 mRNA phenotypes in CRCs and their adjacent normal tissue (ANT). Also, a smaller proportion of CRCs (42% and 35.5%, respectively) and their ANTs (36% and 20%, respectively) exhibited low mRNA levels of hMLH1 and hMSH6.

Statistical analysis by Pearson test, showed a correlation between mRNA expression ratios of hMSH2 and hMLH1, in CRCs (r=0.574765736) and ANTs (r=0.618906296), that was more intense in males (r=0.796820522), in stage I (r=0.99204665) or in stage II (r=0.79450379), as well as in tumors without lymph node metastasis (r=0.79405972) (Table 2). Also, a significant correlation was observed between mRNA levels of hMSH6 and hPMS2 in tumors of female patients (r=0.8263588), in stage I (r=0.99792412) or stage II (r=0.96520875), and in tumors without lymph node metastasis (r=0.63185688) (Table 2). Additionally, a correlation was observed between mRNA ratios of hMLH1 and hPMS2 and between hMSH2 and hMSH6, in stage III tumors (r=0.819228481 and r=0.89391764, respectively) and in tumors metastatic to lymph nodes (r=0.81809858 and r=0.89216115, respectively).

**hMSH2 & hMLH1 & hMSH6 & hPMS2 mRNA relative expression**

Calculation of CRCs/ANTS MMR mRNA ratios from quantification
data (Table 1) revealed reduced mRNA expression ratios (≤0.8) of hMSH2, hMLH1, hMSH6 and hPMS2, with 41% (12/29), 45% (14/31), 53% (16/30) and 36.7% (11/30), respectively. As well as we observed overexpression (CRCs/ANTS ratios ≥1.8) of hMSH2, hMLH1, hMSH6 and/or hPMS2 in 41% (12/29), 32% (10/30), 36.7% (11/30) and 32% (10/30) of cases, respectively (Table 1).

There was a statistically significant correlation between relative expression levels of hMSH6 and hMLH1 for hMSH2, hMLH1 and hMSH6 mRNA expression ratios (≤0.8) of cases without lymph node metastasis (r=0.999477) and also, a correlation was observed between hMLH1 expression levels (T/ANT) of CRCs with lymph node metastasis, perineural or vascular invasion (p<0.009, p=0.000 and p=0.000, respectively) relative to cases without tumor invasion. Also, r was more frequent in CRCs located on the right colon or rectum compared to sigmoid colon tumors (p=0.000) (Figure 1 and 4).

Moreover, r was more common in CRCs compared to their ANTs (p<0.002, χ²-test), mainly in males (p=0.000). A significant difference was found between male and female patients (p<0.02; χ²-test). Also, r was more often in CRCs of stage II and III relative to I (p<0.0002, respectively) and CRCs of stage III relative to II (p<0.01). Nevertheless, the r was more common in CRCs with lymph node metastasis (p<0.004) or nerve (p<0.002) or vessel infiltration (p<0.0034) compared to their ANTs, with statistically significant difference between the number of cases showed nerve or vessel infiltration and those did not (p<0.0045 and p<0.01, respectively). In addition, r was more frequent in CRCs of rectum (p<0.000) relative to their ANTs with a statistically difference relative to sigmoid or right colon (p<0.03) (Table 3, Figure 2 and 4).

Additionally, r was observed commonly in ANTs of female patients relative to their CRCs (p<0.000), with a significant difference between female and male (p<0.009). However, r found commonly in stage III CRCs (p<0.0068) relative to their ANTs. A significant difference of r phenotype was found between stage III and stage II (p<0.0042) or I (p=0.000) as well as between stage II and I (p=0.000) cases. So, r was more frequently observed in CRCs with lymph node metastasis (p<0.002) relative to their ANTs, with a significant difference between number of cases found with lymph node or nerve or vessel infiltration (p=0.000, p=0.000 and p=0.002, respectively) and those without tumor infiltration. In addition, r was more frequent in CRC tumors located to rectum relative to right colon (p=0.000) or to sigmoid (p=0.000) (Table 3, Figure 3 and 4).
Distribution of combined MMR mRNA phenotypes in CRCs and their ANTs

Table 4 presents the distribution of combined MMR mRNA in CRCs relative to patients' and tumors' characteristics. We found a statistically different distribution of \( r_{2r1}, r_{2R1} \) and \( R_{2R1} \) phenotypes i) between CRCs and ANTs in male (p<0.03), ii) between male and female patients (p<0.03), iii) between CRCs and ANTs in stage III cases (p<0.026), iv) between stage III and stage II cases (p=0.023), v) between CRCs and their ANTs in cases with lymph node metastasis (p<0.02), vi) between cases with lymph node and without tumor metastasis (p<0.0014), vii) between CRCs and their ANTs of cases with perineural invasion (p<0.01), viii) between cases with nerve and without nerve invasion (p=0.000) and ix) between cases presented vessel invasion relative to cases without vessel invasion (p=0.000). Specifically there was a significant different distribution of \( r_{2r1} \) and \( r_{2R1} \) phenotypes between CRCs and ANTs in cases located on rectum or sigmoid (p=0.01) relative cases on right colon (p=0.000) and p=0.01, respectively).

The distribution of combined MMR mRNA phenotypes \( r_{2r6}, r_{2R6} \) and \( R_{2R6} \) was statistically significant different i) between CRCs and ANTs in male (p=0.000), with a difference between male and female patients (p<0.03), ii) between stage III and stage II cases (p=0.023), iii) between CRCs and their ANTs in cases with lymph node metastasis (p<0.02), iv) between cases with lymph node and without tumor metastasis (p<0.0014), vii) between CRCs and their ANTs of cases with perineural invasion (p<0.01), viii) between cases with nerve and without nerve invasion (p=0.000) and ix) between cases presented vessel invasion relative to cases without vessel invasion (p=0.000). Specifically there was a significant different distribution of \( r_{2r1} \) and \( r_{2R1} \) phenotypes between CRCs and ANTs in cases located on rectum or sigmoid (p=0.01) relative cases on right colon (p=0.000) and p=0.01, respectively.)
(p=0.000), ii) between CRCs and ANTs in stage III CRCs (p<0.027), with a difference between stage III and stage II (p=0.05) or between stage III and stage I (p=0.000) cases iii) between cases with node metastasis and cases without node infiltration (p<0.002), iv) between CRCs and their ANTs in cases with perineural invasion (p=0.04), v) between cases with perineural invasion compared to cases without perineural invasion (p=0.000) and v) between CRCs and ANTs in vessel filtrated cases (p=0.000), with a difference between vessel filtrated cases and non-vessel filtrated (p<0.005) (Table 4).

The distribution of $r_1^R$, $r_1^R$, and $R_1^R$ was significant different between CRCs and ANTs in stage III cases (p<0.02), with a difference between Stage III and Stage II (p<0.002), and between node (p<0.0024), nerve (p<0.001), vessel (p=0.000) filtrated cases and cases without filtration (Table 4).

**Discussion**

The aim of this study was to quantify the mRNA levels of hMSH2, hMLH1, hMSH6 and hPMS2, MMR genes in sporadic colorectal carcinomas and their ANTs, by Q-real time PCR and to correlate with clinical and histopathological data. So far, there is limited information on the transcriptional levels of the four major MMR DNA repair mechanism components, in non-hereditary CRCs and their ANTs [25,26]. Despite of the small number of patients [31] included in this study our results could be considered interesting and could give rise in a more extended investigation. We showed that the crucial components of MMR mechanism hMSH2 and hMLH1, and their counterparts hMSH6 and hPMS2 exhibited low mRNA expression profiles in a significant proportion of CRCs and their ANTs (58-100%). We also showed that mRNA expression correlated with tumor progression and tumor localization in the colon. Additionally, the transcription of the studied MMR genes was reduced in a significant proportion of CRCs (37-53%) relative to their paired ANTs indicating a possible mechanism of progressive genetic instability [1-3]. We have evaluated various MMR mRNA expression profiles and their relationship to tumor or patients' characteristics.
We observed that CRCs of early histopathological stages (I-II), without lymph node metastasis, exhibited a correlation between the expression status of crucial MMR components, hMSH2 & hMLH1, and between their counterparts hMSH6 & hPMS2, maintained both in CRCs and in their ANTs [27]. Notably, this correlation was reversed between male and female. Surprisingly, cases with lymph node metastasis, revealed a significant positive correlation of mRNA relative expression between hMSH2 and its counterpart hMLH6. Similarly, late stage (III) CRCs exhibited correlation between mRNA expression levels of hMLH1 and its counterpart hPMS2. Our results show a balanced transcriptional activation between the crucial MMR components hMSH2 & hMLH1 and between their counterpart’s hMSH6 and hPMS2, in CRCs of early stages related to gender. Surprisingly, later, an upcoming “unbalance” occurs reducing similarly the mRNA expression levels of MutLa or MutSa components in CRCs relative to their ANTs, that seem to related to tumor progression. Our observations could be significant, indicating a mechanism resulting to downregulation of hMSH2 and hMSH6 expression during tumor progression. The reduction of mRNA expression of MMR genes has been considered to be caused by gene deletions in hereditary cancers, like Lynch syndrome, or epigenetic modification of genes like methylation of hMLH1 in sporadic cancers or as recently it has been shown by specific mRNA regulation [28-34].

Cell biological studies have been shown the importance of retainance of MutLa components balance on cell cycle progression or apoptosis procedure, showing that the MutLa protein levels are essential to initiate apoptosis and consequently low expression levels lead to chemo-resistance [35,36].

Phenotypic sorting of our data revealed that the reduced \( r_2 \) phenotype of crucial hMSH2, MMR mechanism component, was very common both in CRCs and their ANTs indicating a deficiency of MMR mechanism in epithelium of CRCs patients. Significantly, the reduced \( r_2 \) phenotype of hMSH6, the counterpart of hMSH2, was more frequently observed in CRCs relative to their ANTs, supporting an affected MMR mechanism in CRC patients. Moreover, reduced \( (r_2, r_3) \) mRNA MMR phenotypes are related with tumor invasion, indicating their use as a tumor progressing index. We first observed that the reduced \( p_2 \) phenotype of hPMS2 is a common finding in colorectal epithelium of patients with CRC. It is worthy to mention that hPMS2 low expression levels have been previously related with hereditary cancers with a late tumor onset [37]. Here, we suggest that tumorigenesis in colon could be probably related with a molecular mechanism including decreased transcriptional activity of hPMS2.

Observing the combined phenotypic sorting of our data, we can summarize that reduced \( r_2, r_3, r_4 \) combined MMR phenotypes were related to advanced tumors (stage III) and gender. Specifically, \( r_2 \) commonly shows a strong correlation with tumors presented with lymph or nerve or vessel invasion as well as \( r_2 \), with nerve or vessel infiltration. In our previous study in lung cancer we suggested that \( r_2 \) could be considered as a tumor progression index, while it has been correlated with worst prognosis in squamous cell lung carcinomas. Our data suggests that it could be an indicator of tumor progression in CRC and is in agreement with previous findings [16]. Also, low MMR phenotypic profiles are correlated with male gender.

In conclusion, we presented for the first time a precise quantification of MMR mRNA levels, of hMSH2, hMLH1, hMSH6 and hPMS2, in small number of sporadic CRCs and their ANTs, correlated with clinical and histopathological data. Our findings indicate that tumoral epithelium of CRC patients of our group acquires MMR deficiency, during tumor progression. Distinct MMR mRNA profiles as low hMSH2, hMLH1 or hMSH6 mRNA levels \( (r_2, r_3, r_4, r_5) \) could be characterized as important indicators of lymph node metastasis and of perineural or vascular invasion. A different expression pattern was found in males related to females with males showing MMR mRNA profiles related with tumor progressing. All CRCs and their ANTs of our cohort revealed low hPMS2 mRNA levels that were previously correlated with late tumor onset on hereditary colon cancers [37]. Rectal localization was related with dysregulated MMR mRNA mechanism.

References

19. Burger M, Denzinger S, Hammerschmid CG, Tannapfel A, Obermann EC,


34. ERS annual congress Barcelona, Spain 2013. Vageli D, Doukas SG, Kerenidi T, Koukoulis GK, Gourgoulianis KI and Daniil Z (2013) Correlation of miR-422a, miR-21 and miR-155 analysis with hMSH2 and hMLH1 mRNA expression profiles in non-small cell lung carcinomas and their adjacent normal tissues. ERS annual congress Barcelona, Spain.

