

Mismatch Repair and Multi-Drug Resistance Genes Expression in Relation to HCV-Associated Hepatocellular Carcinoma

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ABSTRACT

DNA mismatch repair (MMR) is an important mechanism involved in maintaining fidelity of genomic DNA. Defective MMR is implicated in a variety of tumors; however, its role in hepatocellular carcinoma (HCC) has not been determined yet. Using a newly developed multiple reverse transcription-PCR assay, expression of four of the human MMR genes (hMLH1, hPMS1, hPMS2, GTBP6) and MDR-1 gene in 23 human HCC cases as well as 7 normal adjacent liver tissue (NALT) were evaluated. Twenty-one cases were associated with HCV infection. This was done in an attempt to determine the role of MMR genes in the development of HCC and their possible association with the acquisition of drug resistance. The β -actin gene was used as an internal control for RNA degradation and DNA contamination and as a reference for quantifying the levels of their transcription. Out of the 23 HCC samples, 21 (91.3%) showed reduced expression of at least one of the MMR genes. Low expression of hMLH1, GTBP, hMPS2 and hMPS1 were found in 61.9%, 56.5%, 47.8% and 9.0% of the cases, respectively. MDR-1 expression was detected in 14 out of 23 cases (60.9%). Univariate analysis showed a significant correlation between reduced expression of hMPS2 and GTBP6 ($p = 0.003$), hMPS2 and chronic active hepatitis ($p = 0.05$) as well as hMPS2 and cirrhosis ($p = 0.05$); whereas, multivariate analysis showed that hMPS2 explains 31% and GTBP6 explains 19% of HCC associated with cirrhosis. On the other hand, 83.3% and 28.5% of the NALT showed low expression of hMLH1 and GTBP6, respectively; while 14.2% showed low expression of hMPS2 and hMPS1 (each) and 42.8% showed MDR-1 expression. In conclusion, this study represents the first report illustrating the implication of MMR genes in HCV-associated HCC and their association with MDR-1 overexpression. It also shows that MMR defects occur at an early stage of hepatocarcinogenesis as reduced expression of some MMR genes was detected in cases of chronic active hepatitis (CAH) and cirrhosis. This has a clinical impact, since these genes could be used to monitor CAH and cirrhotic patients who are prone to develop HCC. However, these findings need to be confirmed in a larger cohort including more samples of HCC, CAH and cirrhotic patients.

Key Words: HCC - MMR - HCV.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [11]. It represents 20-40% of human cancers in countries endemic for viral hepatitis. It was found that most of the HCC cases in these areas are late complications of chronic viral hepatitis [22]. The major risk factors for the development of HCC are well defined now and several etiologic factors have been determined including chronic viral hepatitis, alcohol and metabolic disorders. These factors induce malignant transformation by increasing cellular turnover as a consequence of chronic liver injury, regeneration and cirrhosis. However, the exact mechanisms and sequence by which these factors interact at the molecular level to produce HCC have not yet been determined [30].

The DNA mismatch repair system (MMR) is expressed in all tissues at various levels. Biochemical and genetic studies in eukaryotes have defined at least 5 genes (MSH2, MSH3, GTBP6, MLH1 and PMS2) whose protein products are required for DNA-MMR [16]. The MMR system plays an important role in the maintenance of genomic integrity as it corrects replicative mismatches that escape DNA polymerase proof reading [7]. Direct evidence for the association of genetic instability and mutant mismatch repair genes is derived from the biochemical studies in vitro in which nuclear extracts from human tumor cell lines with mutated MMR genes were unable to efficiently repair heteroduplex DNA fragments [27]. Hence, it was mentioned that cells with defective MMR mechanisms have a reduction in the fidelity of

DNA and cannot correct genetic errors that occur during cellular replication [19].

Recent studies have focused on the impact of defective MMR genes on the pathogenesis of malignancy and genes encoding components of the MMR system have been mentioned in relation to several human solid tumors [12,32&39]. Benchenhou et al. [3] mentioned that, defects in mismatch repair genes lead to a genome-wide instability of microsatellites which are sequences of the DNA comprising multiple copies of repeat units 1-6 base pairs [3]. When this occurs in oncogenes or tumor suppressor genes, loss of control over cell growth and proliferation may develop [17].

Microsatellite instability (MSI) was reported in many solid tumors, however the role of such defects in HCC has not yet been elucidated due to the great variability in the frequency of MSI and LOH in different studies [34&35]. The exact time of occurrence of such defects is of much concern especially in HCV-associated HCC, which is usually preceded by several stages of virus-induced hepatic damage.

It was shown that loss of expression of the mismatch repair genes leads to resistance of tumor cells to the damage induced by some chemotherapeutic agents. In addition, loss of DNA-MMR activity causes high level resistance to the antimetabolite 6-thioguanine [10], moderate resistance to some methylating agents [15] and low level resistance to cisplatin and carboplatin [1&8]. This acquired resistance could be achieved through several mechanisms such as failure to recognize DNA adducts formed by some chemotherapeutic agents (MMR), failure to activate signaling pathways that trigger apoptosis [7] or through other unidentified pathways.

Overexpression of either the multidrug resistance gene MDR-1 and/or multidrug resistance protein (MRP) have been shown in many studies on HCC to be associated with resistance of tumors to multiple chemotherapeutic agents [5&23]. Expression of P-glycoprotein has been observed in 52-95% of HCC cases. Moreover, patients with P-glycoprotein positive tumors have shown shorter disease-free and overall survival rates than those with P-glycoprotein negative tumors [5&29].

Therefore, this work was conducted to: 1- Determine the role of MMR gene defects in the development of HCV associated HCC, and 2- Investigate the proposed relationship between defective MMR genes and the expression of MDR-1 gene as one of the possible mechanisms involved in the acquisition of drug resistance associated with defective MMR genes.

PATIENTS AND METHODS

Tumor samples: In the present study, a total of 30 samples were analyzed including 23 cases of HCC and 7 cases of morphologically normal liver tissues adjacent to malignant tumors. All samples were obtained from patients undergoing hepatectomy at the National Cancer Institute, Cairo University during the period 1999-2001. The age range was 30-80 years (mean = 49) and the male : female ratio was 1.1:1. The pathological details of the tumors including the presence of cirrhosis, chronic active hepatitis and HCV infection as well as the clinical stage are illustrated in Table (1). Fresh tumor and normal tissues were obtained at operation and divided into two pieces. One fragment was immediately snap frozen and stored at -70°C for subsequent DNA and RNA extraction and the other was fixed in neutral buffered formalin and processed for histopathologic examination to determine: tumor type and grade, the presence of cirrhosis and/or CAH as well as the percent of neoplastic cells in the specimen. Only tumors in which the neoplastic cells represented 75% or more were included in order to avoid the neutralizing effect of normal cells. Normal tissues were obtained from areas distant to tumors (1-2 cm from the periphery of the tumor) and confirmed to be normal by microscopic examination of H&E-stained sections. Both normal and neoplastic tissues were examined by two independent pathologists. Unintentional bias was prevented by coding patient tissue samples so that genomic studies were done without knowledge of the patient and tumor characteristics. All samples included in this study were HBV-PCR negative.

DNA extraction: High molecular weight DNA was prepared from 0.5-2.0 g fresh tissue samples according to standard protocols [28].

RNA extraction: RNA from both tumor and normal tissues was extracted using SV total RNA Isolation System (Promega Biotech). The extracted total RNA was assessed for degrada-

tion, purity and DNA contamination by spectrophotometry and electrophoresis in an ethidium bromide-stained 1.0-% agarose gel.

c-DNA synthesis: Reverse transcription of the isolated total RNA was done in 20 μ l reaction volume as previously described [33]. c-DNA from 10 normal blood donors were used as positive control in the initial PCR reaction to test for the specificity of the primers and to adjust the PCR conditions. In addition, normal human DNA was used to optimize the best conditions for the multiplex PCR of the β -actin gene versus each of the studied genes (Fig. 1). Amplification of B-actin gene (621-bp fragment) was performed to test for the presence of artifacts as well as to set a baseline for each sample that enables the evaluation of the expression levels of target genes in the multiplex RT-PCR (i.e. quantitation). The β -actin fragments were also used to monitor DNA contamination. Because all genes were amplified in the same test tube, designing only one pair of primers should be sufficient for controlling DNA contamination. For example, the 612-bp fragment of B-actin spans the exon 3/intron 4 (95 bp)/exon 4/intron 5 (112 bp)/exon 5 boundaries. If there was cDNA contamination with genomic DNA, the PCR product would contain 828-bp band that contains introns 4 & 5 and could be distinguished on a 2% agarose gel. A water control tube containing all reagents except cDNA was also used in each batch of PCR assays to monitor for contamination of genomic DNA in the PCR reagents.

PCR amplification for the MMR genes: The PCR and quantitation were performed in a 50 μ l reaction volume as described by Wei et al. [33] with some modifications. Briefly, 2.5 units Taq polymerase (Gibco-BRL, Gaithersburg, MD, USA), 1XPCR buffer containing 500 mM KCL, 200 mM Tris-HCL, 1.5 mM MgCL₂, bovine serum albumin (BSA) 1 mg/ml, 10 μ l of the RT reaction mixture (c-DNA), 200 μ M each of the deoxyribonucleotide triphosphate and 2 μ M of each primer were mixed. Samples were denatured at 95°C for 5 min and subjected to 35 rounds of thermal cycling (T-gradient-Biometra-Germany). Each cycle consisted of denaturation for 1 min at 95°C, annealing for 1 min at different annealing temperatures as shown in Table (2) and extension for 1 min and 30 sec at 72°C. Samples were then incubated for 10 min at 72°C. All samples were analyzed twice for

MMR by the RT-PCR on different days to ensure reproducibility of results.

PCR amplification for the MDR-1 gene: The RT enzyme was inactivated by incubation at 72°C for 5 min and PCR amplification was performed in 50 μ l reaction volume as described by Zhou et al. [43]. After completion of the amplification reaction, 15 μ l of each PCR reaction product were analyzed by electrophoresis in a 2% ethidium bromide-stained gel. Samples were analyzed twice for MDR-1 by the RT-PCR on different days to ensure the reproducibility of the results.

Quantitation: Fifteen microliters of each reaction product were separated by electrophoresis through a 2.0% ethidium bromide stained agarose gel and visualized with ultraviolet light. Gels were video-photographed; the bands on the photograph were scanned as digital peaks and the areas of the peaks were calculated in arbitrary units with a Digital Imaging System (Model IS-100; Alpha Innotech Co., San Leandro, CA, USA). to evaluate the relative levels of expression of the target genes in multiplex RT-PCR, the value of the internal standard (β -actin) in each reaction was used as a normalizing factor and a relative value was calculated for each target gene amplified in the reaction. Reduced expression in any of the studied gene was considered if there is complete absence or decrease in the intensity of the desired band more than 75% compared to the band of the β -actin gene expression. On the other hand, over-expression of the MDR1 gene was scored positive when showing a band of any intensity at the right position at 242 bp. Samples were assayed in batches including both cases and controls. The absence of bands was verified by repeating the multiple PCR assays.

RT-PCR of HCV: The RT and PCR were performed as described by Zekri et al. [40]. After completion of the reaction, 10 μ l of each sample were analyzed by electrophoresis through a 1.2% ethidium bromide-stained agarose gel and DNA was transferred from the gel onto a nitrocellulose filter with alkaline buffer (4N NaOH). The transferred DNA was cross-linked by incubation for 2-3 hours at 80°C and the blot was then hybridized with an internal probe.

Detection of HBV-DNA in tissues: PCR amplification of HBV was done as previously de-

scribed by Boom et al. [4] and then, 10 μ l of each reaction product were analyzed by electrophoresis through an ethidium bromide-stained 1.2% agarose gel.

Statistical analysis: All the data were analyzed using SPSS 9.0 for Windows. Wilcoxin's rank test and simple correlation were first used to determine preliminary significant relations available. However, since this type of study is the first to be done in the region, more in-depth analysis was then employed using multivariate analysis for more accurate significant results.

RESULTS

Out of the 23 studied HCC cases, 21 (91.3%) showed reduction in the expression of one or more of the 4 studied MMR genes (hMLH1, GTBP6, hMPS1 and hMPS2) but none showed simultaneous reduction in the 4 genes. The most commonly affected gene was hMLH1, where reduced expression was detected in 13/21 cases (61.9%), followed by GTBP6 (13/23 cases representing 56.5%), hMPS2 (11/23 cases representing 47.8%) and hMPS1 (2/22 cases representing 9%). However, 8/23 cases (34.8%) had reduction in both GTBP6 and hMPS2, 7/22 cases (31.8%) had reduction in both GTBP6 and hMLH1 and 6/21 cases (28.5%) had reduction in both hMPS2 and hMLH1. There was a statistically significant correlation between reduced expression of GTBP6 and hMPS2 ($p = 0.003$) but not between GTBP6 and hMLH1 or hMPS2 and hMLH1 (Figs. 1&2).

Using multivariate analysis between HCC and the 5 studied genes, GTBP6 significantly explains 19% of the frequency of HCC ($p = 0.06$); whereas, hMPS2 explains 31% of the frequency of cirrhosis in HCC cases ($p = 0.001$). MDR-1 gene expression was detected in 14/23 (63.6%) cases of HCC and in 3/7 (42.8%) of the NALT samples and it significantly explains 14% of the frequency of cirrhosis ($p = 0.09$).

Relation between MDR-1 and the studied MMR genes: The correlation between MDR-1 gene expression and the 4 studied MMR genes revealed that the MDR-1 positive cases showed reduction in 9/14 (64.2%), 8/14 (57.1%), 7/14 (50%) and 1/14 (7.0%) of GTBP6, hMPS2, hMLH1 and hMPS1 genes, respectively. Four cases had reduction in both GTBP6, hMPS2

and hMLH1 of which 3 had detectable MDR-1 gene expression.

The relation between the 5 studied genes and HCC in the presence of cirrhosis: Reduced expression was seen in 5/11 patients with cirrhosis (45.4%) compared to 8/12 (66.6%) cases without cirrhosis for GTBP6, in 1/0 (10%) cases compared to 1/12 (8.3%) cases for hMPS1, in 2/11 (18.1%) compared to 9/12 (75.0%) cases for hMPS2 and in 6/10 (60%) compared to 6/11 (54.5%) for hMLH1. There was a statistically significant difference between normal and tumor samples in the expression level of hMPS2 ($p = 0.05$), while no significant relation was obtained with GTBP6.

MDR-1 gene expression showed detectable levels in 7/10 (70%) patients with cirrhosis compared to 7/12 (58.3%) patients without cirrhosis ($p = 0.014$).

The ANOVA test was used to determine the relationship between the 5 studied genes and HCC in presence of cirrhosis as well as the relation between these genes and the clinicopathologic features of the patients. The only significant relations were found between reduced expression of hMPS2 and HCC on top of cirrhosis ($p = 0.015$) as well as between hMPS2 and high tumor grade ($p = 0.0001$).

The present study included 4 cases of CAH without cirrhosis. All these cases showed reduction in hMPS2 level, 2 showed reduction in GTBP6 level and 2 showed reduction in hMLH1 level. There was a trend for cases with CAH to have reduced expression levels of hMPS2. However, no statistical analysis was performed due to the small sample size.

The NALT samples: The expression level of GTBP6 was reduced in 2/7 (28.5%) samples of NALT compared to 56.5% for HCC samples, in 1/7 (14.2%) NALT compared to 9% of HCC for hMPS1, in 1/7 (14.2%) NALT compared to 47.6% of HCC cases for hMPS2 and in 5/6 (83.3%) NALT compared to 61.9% of HCC samples for hMLH1. Out of the 3 cases that had detectable levels of MDR-1 in NALT, one case showed reduction in hMPS2, one in both hMPS2 and hMLH1 and one in GTBP6, hMPS1 and hMPS2 (Fig. 3).

A statistically significant difference was observed between cases of HCC and NALT in the expression level of hMPS2, GTBP6 and hMLH1.

Table (1): Clinical feature of the studied patients.

Serial No.	Age	Sex	Diagnosis	Grade	Cirrhosis	CAH	HCV	HBV
1	30	F	HCC	II	-	-	+	-
2	50	F	HCC	II	-	-	+	-
3	50	F	HCC	II	-	-	-	-
4	51	F	HCC	II	-	-	+	-
5	80	M	HCC	III	-	-	+	-
6	65	M	HCC	III	-	-	-	-
7	57	M	HCC	III	-	-	+	-
8	55	M	HCC	II	-	-	+	-
9	35	F	HCC	II	-	+	+	-
10	51	M	HCC	II	-	+	+	-
11	50	F	HCC	III	-	+	+	-
12	60	F	HCC	II	-	+	+	-
13	58	F	HCC	I	+	-	+	-
14	10	F	HCC	II	+	-	+	-
15	68	M	HCC	II	+	+	+	-
16	55	M	HCC	II	+	-	+	-
17	63	M	HCC	III	+	+	+	-
18	56	M	HCC	III	+	-	+	-
19	63	M	HCC	II	+	-	+	-
20	58	F	HCC	II	+	-	+	-
21	65	M	HCC	III	+	-	+	-
22	48	F	HCC	II	+	-	+	-
23	60	M	HCC	II	+	-	+	-
1	55	M	NAT	-	-	-	+	-
2	63	M	NAT	-	-	-	+	-
3	55	M	NAT	-	-	-	-	-
4	40	F	NAT	-	-	-	+	-
5	40	M	NAT	-	-	-	+	-
6	50	M	NAT	-	-	-	+	-
7	35	F	NAT	-	-	-	+	-

Table (2): Oligonucleotide primers for MMR genes, MDR-1, HCV and HBV.

Gene	Primer sequence	PCR product, bp	Annealing temp (°C)
β -actin	5'-ACACTGTGCCCATCTACGAGG-3' 5'-AGGGGCCGGACTCGTCATACT-3'	621	54-59.9
HMPS2	5'-TGCATGCAGGATTTGGAAA-3' 5'-GAACCCCTCAGAATCCACGGA-3'	385	55
GTBP6	5'-CCCTCAGCCACCAAAGAAGCA-3' 5'-CTGCCACCACTTCCTCATCCC-3'	288	56
HMLH1	5'-GTGCTGGCAATCAAGGACCC-3' 5'-CACGGTTGAGGCATTGGGTAG-3'	215	58
HMPS1	5'-GCGGCAACAGTTCGACTCCTT-3' 5'-AGCCTTGATACCCTCCCCGTT-3'	174	57
MDR-1	5'-CTAATAAGAAAAAGATCAACT 3' 5'-AATCTTTGAAAATATTATTGC 3'	242	55
HBV			
LBL	5'-CGGATCCGTGGAGTTACTCTGGTTTTTGC-3'	460	55
RBL	5'-GCAAGCTCTAACAAACAGTAGTTCCGG-3'		
HCV			
RB6A	5'-GTA AGG AAC TAC TGT CTT CAC G-3' (nt47-68)	266	55
RB6B	5'-ACT CGC AGG CCT ATC AGG (nt 292-312)		

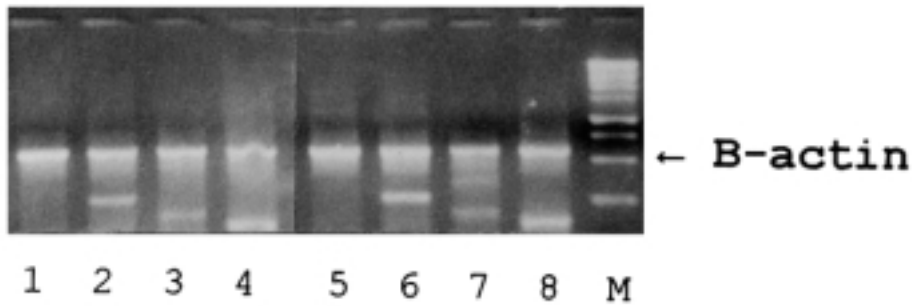


Fig. (1): Multiples RT-PCR amplification of MMR genes in normal PBL. M: Molecular weight marker; lanes 1 and 5 β -actin gene in normal PBL as a control; lanes 2 and 6 GTBP6 co-amplified with β -actin; lanes 3 and 7 hMLH1 co-amplified with β -actin; lanes 4 and 8 hMPS1 co-amplified with β -actin. PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide and visualized with UV.

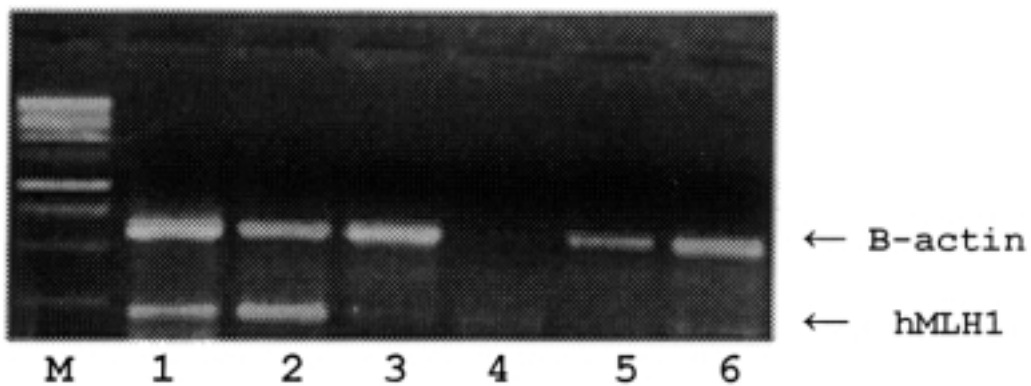


Fig. (2): Multiples RT-PCR amplification of hMLH1 gene in HCC cases. M: Molecular weight marker; lanes 1,2,3,5 and 6 hMLH1 co-amplified with β -actin in HCC cases, lane one showed 50% reduction, lanes 3,5 and 6 showed 100% reduction. Lane 7 hMLH1 co-amplified with β -actin gene in normal PBL as a control; lane 4 hMLH1 co-amplified with β -actin without the RT enzyme as a RT-PCR control. PCR products were electrophoresed in 2% agarose gel stained with ethidium bromide and visualized with UV.

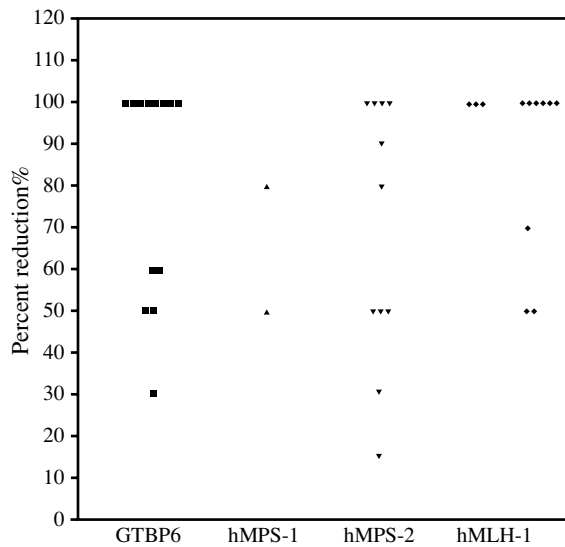


Fig. (3): A scatter diagram represents the percent reduction of the 4 studied MMR genes in each of the studied HCC cases.

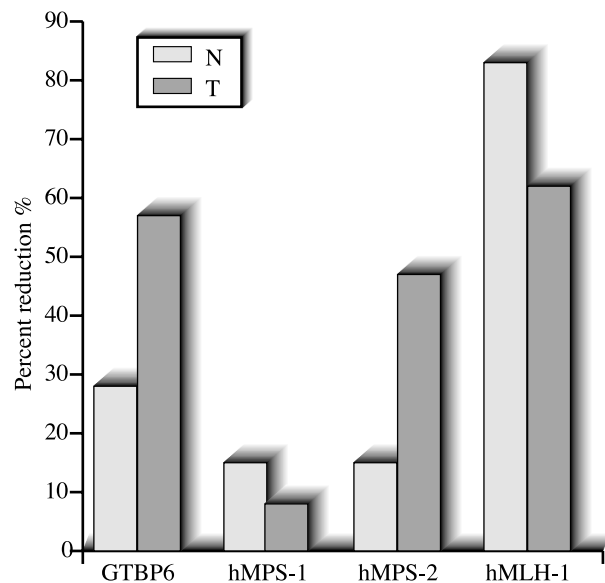


Fig. (4): Histogram showing the frequency of the percent reduction of the 4 studied MMR genes in HCC cases compared to the normal adjacent tissue.

DISCUSSION

So far, only very few reports were published regarding the role of MMR genes in the pathogenesis of HCC [34-36]. In the present study, we used a newly developed multiplex RT-PCR assay to investigate the role of some MMR genes (hMLH1, GTBP6, hMPS1 and hMPS2) in hepatocarcinogenesis and their possible association with MDR-1 gene expression. We demonstrated that reduced expression of hMLH1, GTBP6 and hMPS2 is frequent in HCC patients suggesting a possible role for these genes in the hepatocarcinogenesis. On the other hand, reduced expression of hMPS1 was relatively infrequent. The results of the few studies performed in this context are contradictory making the comparison between them a difficult task. So, whereas Yamamoto et al. [35] found that widespread MSI due to defective MMR genes played a little role, if any, in hepatocarcinogenesis, Yano et al. [36] demonstrated that mutations of hMSH2 closely correlated with survival of HCC patients and that defective DNA mismatch repair had a significant role in hepatocarcinogenesis. Also, Yakushiji et al. [34] demonstrated in a case report that abnormal DNA-MMR played a major role in the occurrence of multiple primary cancers including HCC. However, this contradiction could be explained by the difference in the studied genes and the variable mechanisms by which these genes could be inactivated. In the present work, we studied the impact of reduced expression of MMR genes. However, other mechanisms of gene inactivation might be involved such as mutations, LOH, deletion or hypermethylation [6&20].

A striking finding in this study was that reduced expression of hMLH1 was more frequent in NALT than in tumor tissues, in cases from which NALT were obtained. A similar observation was reported in breast cancer cases and the authors explained their finding by assuming that the area from which NALT was obtained, was morphologically but not genetically normal [42]. The fact that, the majority of our cases were HCV positive could provide another explanation for such finding as well as for the reduced expression of all other studied genes in NALTs. It could be assumed that the virus may have a role in dysregulating the expression of these genes, but this needs to be confirmed in a larger study on HCC in HCV infected patients.

Our results regarding the reduced expression level of MMR genes in NALT are comparable to Macdonald et al. [19] who have detected LOH at hMSH2 and/or hMLH1 in malignant, premalignant and adjacent hepatic tissues providing evidence for the early implication of these genes in HCC. This also confirms the previously mentioned assumption of Zekri et al. [42] that the area adjacent to tumor may harbor some genetic aberrations that facilitate local spread and recurrence.

Our observation regarding the high incidence of reduction in GTBP6 level made this gene a possible candidate for HCC. This was further confirmed by the observation that in all cases, which showed reduced expression of more than one gene, GTBP6 was always a partner. On the contrary, studies performed on other solid tumors, e.g. gliomas, did not reveal a significant role for this gene [32] indicating that defects in the MMR gene family could be tumor specific since abnormalities of certain MMR genes were restricted to certain tumor types [14&31].

HMPS2 was the second most frequently affected gene, especially in cases of HCC arising on top of cirrhosis. Moreover, a statistically significant correlation was reported in this study between hMPS2 and cirrhosis. This clearly indicated that this gene played an important role in the development of cirrhosis in HCV infected patients and may subsequently lead to neoplastic transformation of hepatocytes. In addition, the significant association between reduced expression of GTBP6 and hMPS2 in HCC, especially in cases with cirrhosis, could be explained by the presence of a cooperation between these two genes in the very early stages of hepatocarcinogenesis.

A striking finding in this study was that, although the number of CAH cases analyzed was so small, all these cases showed reduced expression of hMPS2 gene. Again, this finding might indicate that hMPS2 could be the first MMR gene to be affected in the genetic cascade controlling hepatocarcinogenesis in HCV - infected patients.

It has been previously mentioned that defects in MMR genes can induce resistance to certain chemotherapeutic agents and that, although sometimes this resistance is of a relatively small degree, it is of biological and clinical

cal significance [7]. Many authors have shown that enhanced DNA repair capacity may contribute to drug resistance in some cell lines [37,38&44]. However, the molecular mechanisms of altered DNA repair in drug-resistant cells as well as their relevance to the situation in vivo are still unknown.

Loss of DNA-MMR can have an impact on the responsiveness of a tumor to chemotherapy in several ways. *First*, loss of MMR leads to inability to remove adducts formed by certain chemotherapeutic agents [18]. *Second*, it has been previously reported that HCV-associated HCC involves alterations in the concerted action of protooncogenes, growth factors and tumor suppressor genes. The presence of 2 nuclear localization signals and a DNA binding motif in the HCV core protein suggest a possible functional role for HCV as a gene regulatory element [41]. Moreover, some studies suggest that this protein interacts with certain cellular proto-oncogenes at the transcriptional level, resulting in the promotion of cell proliferation and thus affecting normal hepatocyte growth. Therefore, the pathogenesis of HCV may be attributed, at least in part, to the upregulation of hepatocyte growth induced by HCV core protein [26].

Numerous studies have shown that overexpression of growth factor receptors is associated with altered cellular response to DNA damage and DNA repair. Zing-rong and colleagues [24] have noticed that, there is a direct correlation between overexpression of c-erbB-2 product, drug resistance and elevated DNA repair capacity in lung cells [9], whereas, in breast and ovarian cells over-expression of cerbB-2 gene product has increased sensitivity to drugs through inhibition of DNA repair [2,9&25]. It has been clearly indicated that modulation of cerbB-2 inhibits DNA repair either directly or indirectly through mechanisms such as cell cycle checkpoint [13]. *Third*, genomic instability that accompanies loss of MMR increases the mutation rate in the coding or regulatory sequences of other genes whose protein products may be critical in determining tumor cell sensitivity to drugs [7]. MDR-1 might be one of these genes since overexpression of multi-drug resistance protein (MRP) has been reported in cell lines derived from human HCC [21] as well as in tumor samples [5&23].

In the present study, MDR-1 gene expression was detected in 63.6% of HCC cases, which is comparable to other reports in this context. Ng et al. [23], Chou et al. [5] and Soini et al. [29] reported MDR-1 overexpression in 92%, 52% and 63% of their HCC cases, respectively, using IHC techniques. In addition, Chou et al. [5] showed that, this protein expression was significantly associated with non-responders. However, to our knowledge this study represents the first report on the correlation between MMR genes and MDR-1. According to the results of this work, we propose that the drug resistant phenotype that is associated with defective MMR could be achieved through the overexpression of the MDR-1, since we found a strong correlation between reduced expression of GTBP6, hMLH1, hMPS2 and MDR-1 expression. We also noticed that the expression of MDR-1 gene is higher in cirrhotic than in non-cirrhotic cases indicating that HCC developing on top of cirrhosis is expected to be more resistant to chemotherapy than HCC without cirrhosis. This has a clinical implication since most cases of HCV-associated HCC develop on a background of cirrhosis.

In conclusion, the present study represents a step forward for understanding the genetic events that induce HCC in HCV infected patients. We herein show that GTBP6 is a possible candidate for HCC in HCV infected patients and that reduced expression of hMPS2 occurs in the very early stages of hepatocarcinogenesis. Reduced expression of the studied MMR genes is also associated with the overexpression of MDR-1 gene especially in cirrhotic patients providing evidence that, at least in some tumors, defective DNA repair could induce drug resistance through increasing the expression of P-gp. However, these findings need to be confirmed in a larger study including more samples of HCC, CAH and cirrhotic patients.

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