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Influence of the human MIF promoter polymorphism on hepatocellular carcinoma prognosis

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ABSTRACT. Hepatocellular carcinoma (HCC) is one of the most common worldwide malignancies. A relative complete diagnosis system for primary carcinoma of liver has already been established, but the surgical prognosis for HCC, which depends mainly on postoperative pathological classification and data of recurrence and metastasis, lacks valid experimental indicators. Macrophage migration inhibition factor (MIF) is related to many cancers; hence, the polymorphism of MIF genes may be associated with the surgical prognosis of HCC. The purpose of this study was to investigate the relationship between polymorphisms of MIF gene promoter 794CATT (MIF-794CATT) microsatellite repeats and HCC surgical prognosis and evaluate the contribution of polymorphism to the prognosis of hepatectomy. Sequencing was used to identify the MIF-794CATT of 241 patients who had been submitted to HCC surgery. These patients were classified into 2 groups: one with MIF-794CATT high-repetitive-sequence genotypes (7/x+8/x) and one with low-repetitive-sequence genotypes (5/5+5/6+6/6). Five indictors were analyzed: average survival times were compared using the *t*-test, and tumor-node-metastasis

staging, recurrence and metastasis, differentiation grade, and survival rate were compared using the chi-square test. The (7/x+8/x) CATT group had 139 patients and the (5/5+5/6+6/6) CATT group had 102. Significant differences were found in the 5 factors (P = 0.000, 0.008, 0.002, 0.000, and 0.003, respectively). Patients with MIF-794CATT$_{5-8}$ low-repetitive-sequence genotypes had better prognosis than those with high-repetitive-sequence genotypes. The polymorphism detection of MIF-794CATT microsatellite repeats is valuable for HCC surgical prognosis.

Key words: Hepatocellular carcinoma; MIF-794CATT; Promoter; Polymorphism

INTRODUCTION

Hepatocellular carcinoma (HCC), one of the most frequently diagnosed malignancies, is now second only to lung cancer as the most common cancer in the world. Globally, more than 600,000 new HCC cases are diagnosed every year, and the 5-year survival rate is as low as 5%. The mortality of liver cancer has become higher than that in any other cancer. In China, HCC is the second most common cause of cancer-related death. At present, the combination of imaging techniques and laboratory examination with α-fetoprotein as an important indicator provides a comprehensive diagnostic system for HCC. The coincidence rate of the preoperative and pathological diagnoses has reached 97.4%. However, HCC prognosis depends mainly on postoperative pathological classification and data of recurrence and metastasis (R&M). The prognosis judgment can be made only after the removal and examination of a tissue specimen. Patients with HCC differ in their prognosis data, and the cause of this diversity remains unknown. Macrophage migration inhibition factor (MIF) is related to many cancers and may be a key indicator for cancer prognosis. Studies have demonstrated that polymorphism of the MIF promoter gene influences expression of MIF and tumor growth. By examining the 794CATT microsatellite polymorphism of HCC patients, we analyzed the relationship of the polymorphism with HCC prognosis and evaluated its contribution to prognosis judgments. This study may help prevent cancer and develop more accurate prognoses.

MATERIAL AND METHODS

Clinical samples

Two hundred and forty-one HCC patients who underwent liver resection between January 2009 and June 2011 were recruited from the Department of Liver and Gallbladder, Third Affiliated Hospital of the Third Military Medical University in Chongqing, China. None of the patients had a medical history of gastrosis, systemic lupus erythematosus, diabetes, rheumatoid arthritis, or inflammatory bowel disease.

DNA extraction

Fasting blood (2 mL) from each patient was kept at -20°C in vacuum blood collection

tubes with anticoagulant. DNA was extracted according to the instruction manual (TaKaRa, Japan) and preserved at -20°C for polymerase chain reaction (PCR) experiments.

Detection of MIF-794 promoter microsatellite repeats

The 196-bp gene segment containing the $CATT_6$ repetitive sequence was first amplified using a PCR technique. The forward primer was 5'-CTATCAGAGACCAAGGACAG-3', and the reverse primer was 5'-CCAGGCATATCAAGAGACAT-3'. Initial DNA denaturation was carried out at 95°C for 10 min followed by 45 s of denaturation at 95°C. Annealing temperature was set at 57°C for 40 s, extension time was 40 s at 72°C, amplification was carried out in 35 cycles, and a final cycle was performed for 7 min at 72°C. The sequences of amplification products were then analyzed.

Statistical analysis

The indicators of carcinoma prognosis [including cancer differentiation grade, tumor-node-metastasis (TNM) staging, survival rate, and R&M] in 2 genotype groups (5/5+5/6+6/6 and 7/x+8/x) were analyzed and compared using the chi-square test. Differences in survival times between the 2 groups were analyzed with a *t*-test. All statistical tests were two-sided. SPSS13.0 was used for data processing, and a difference of $P < 0.05$ was considered significant.

RESULTS

Characteristics of clinic samples

The gene sequences of the 241 HCC patients who had undergone hepatectomy revealed that the MIF-749 promoter microsatellite sequence contains 5-8 CATT or AATG repetitive sequences, as shown in Figure 1.

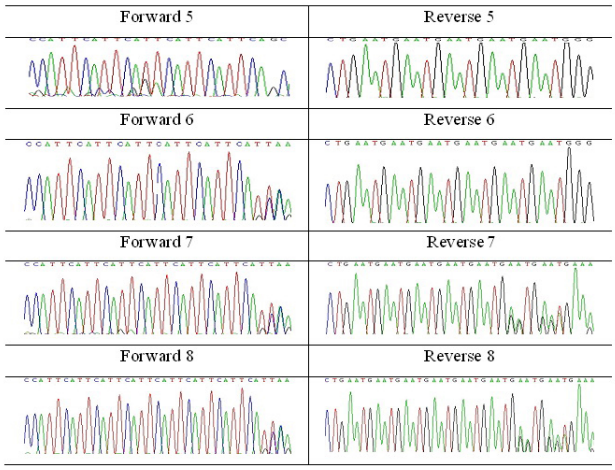

Figure 1. CATT repeats of the -794 microsatellite. 1-4 = sequences obtained with the forward primer. 5-8 = AATG inverted repeats obtained with the reverse primer.

Clinical data of MIF-794CATT$_{5-8}$ genotype patients

The 241 patients in this study had a physical examination and follow-up every 3 months. The longest tracking time was 30 months and the shortest was 6 months (Table 1).

Table 1. Five indicators including average survival time, tumor-node-metastasis (TNM) staging, differentiation grade, recurrence and metastasis (R&M), and survival rate were recorded.

Genotype	Case No. (%)	Average survival time (month)	TNM stage				Differentiation grade			R&M		Survival rate	
			I	II	III	IV	High	Middle	Low	Yes	No	Die	Live
5/5	19 (8)	20.61 ± 7.97	6	6	4	3	4	6	9	7	12	5	14
5/6	32 (13.6)	19.97 ± 8.09	12	7	7	6	4	15	13	15	17	9	23
5/7	25 (10.5)	16.16 ± 7.36	4	7	8	6	2	6	17	14	11	10	15
5/8	4 (1.5)	19.25 ± 8.77	0	1		3	1	0	3	3	1	2	2
6/6	51 (21.0)	18.86 ± 7.40	18	12	13	8	4	21	26	20	31	11	40
6/7	58 (24.1)	15.36 ± 6.28	14	12	16	16	2	8	48	37	21	25	33
6/8	1 (0.4)	21			1				1	1	0	1	0
7/7	25 (10.5)	15.80 ± 7.18	8	3	7	7	2	4	19	15	10	11	14
7/8	26 (10.8)	14.85 ± 6.69	6	4	7	9	2	5	19	16	10	11	15

Prognosis analysis of 5/5+5/6+6/6 and 7/x+8/x genotype patients

The MIF-794CATT genotypes were separated into a high-repetitive-sequence genotype group and a low-repetitive-sequence genotype group (7/x+8/x and 5/5+5/6+6/6, respectively). The statistical results of 5 indicators, shown in Table 2, demonstrated significant difference between the 2 groups ($P < 0.01$).

Table 2. Relationship between the CATT$_{5-8}$ genotype and hepatocellular carcinoma prognosis indicators.

Variable	-794CATT$_{5-8}$ cases		P
	5/5+5/6+6/6	7/x+8/x	
Differentation grade			0.000
High or moderate (%)	54 (22.4)	32 (13.3)	
Low (%)	48 (19.9)	107 (44.4)	
TNM stage			0.008
I + II (%)	61 (27.8)	59 (22.8)	
III + IV (%)	41 (14.5)	80 (34.9)	
Survival rate			0.003
Death number (%)	25 (10.3)	60 (24.9)	
Survival number (%)	77 (32.0)	79 (32.8)	
R&M			0.002
Present	42 (17.4)	86 (35.7)	
Absent	60 (24.9)	53 (22.0)	
Average survival time (months ± SD)	19.46 ± 7.67	15.73 ± 7.68	0.000

Average survival time was analyzed by the *t*-test and others by the χ^2 test. TNM = tumor-node-metastasis; R&M = recurrence and metastasis.

DISCUSSION

MIF, a typical inflammatory corpuscle factor, is not only considered to participate in inflammation and immunoreactions but also regarded as one of the extracellular factors that

promote tumor growth. A large number of studies have indicated that MIF directly influences the division of normal cells and the vicious transformation of oncogenes (Meyer-Siegler et al., 2004), indirectly influences anti-oncogene p53 through immunoregulation, and affects the occurrence and development of tumors through multiple functions, such as promoting the proliferation and migration of cells and angiogenesis (Chesney et al., 1999; Hudson et al., 1999; Shimizu et al., 1999). The human MIF gene is a single-copy gene; its coding gene locates on chromosome 22q11.2 and contains 3 exons and 2 introns. High-performance liquid phase chromosome mapping has shown that the MIF gene displays polymorphism, including microsatellite polymorphism and single nucleotide polymorphism, at 4 sites. G/C polymorphism locates at -173 nt, T/G polymorphism locates at +254 nt, C/G polymorphism locates at +656 nt, and a CATT repetitive sequence locates at -794 nt. Research has also shown that in the MIF-794 region, microsatellite polymorphism, which comes from a CATT 4-nucleotide repetitive sequence, affects MIF expression, which means that the MIF-794 promoter microsatellite polymorphism has a relationship with tumorigenesis and prognosis (Baugh et al., 2002; Radstake et al., 2005). Until now, however, no reports on this relationship have been published.

We detected the gene distribution of the MIF-794 microsatellite region in 241 patients. Four repetitive sequences of $CATT_{5-8}$ were found in these patients. The patients were separated into 2 groups: patients in group 1 had low-repetitive-sequence genotypes (5/5+5/6+6/6), and those in group 2 had high-repetitive-sequence genotypes (7/x+8/x). The survival times of groups 1 and 2 were 19.5 and 15.8 months, respectively, and during the same observation period, patients in group 1 had longer survival times than those in group 2 ($P = 0.0001$). During 30 months of follow-up, the death and survival of group 2 accounted for 24.9 and 32.8% of the total, whereas those in group 1 accounted for 10.3 and 32.0%. The death rate of group 2 was much higher than that of group 1.

Little research on this topic exists worldwide, so a valid comparative analysis was impossible. International studies have indicated that MIF expression has a relationship with postoperative survival. Hira et al. (2005) reported that MIF is related to liver tumor angiogenesis and liver tumor cell migration. Their report also indicated that carcinoma tissues expression is coincident with MIF expression, α-fetoprotein level, and intrahepatic recurrence rate and is especially positively correlated with tumor microvessel density. Furthermore, patients with highly expressed MIF had shorter postoperative survival times. The conclusions of Hira et al. (2005) agree with our results, which demonstrate that the 7/x+8/x genotype group had a shorter survival time. Additional research provides evidence that among the 4 repetitive sequences of $CATT_{5-8}$, the 7/x+8/x gene may increase expression of MIF. Some researchers also believe that MIF expression level depends on the $CATT_{5-8}$ promoter of MIF-173 and -794 together (Donn et al., 2002). We did not prepare 7/x+8/x gene samples, but their examination is the next step in research of MIF expression level in HCC.

Other indicators were also detected in this study. Of the 139 patients in group 2, poor differentiation carcinoma patients accounted to 107, or 44% of the total. Group 1 had 102 patients, in which poor differentiation carcinoma patients accounted to 48, or 19.9% of the total. Significant differences were found in differentiation grade and TNM stage between the groups ($P = 0.000$ and 0.008, respectively). Group 2 had a worse differentiation grade and TNM stage than that of group 1. Owing to a lack of HCC cases, pairwise comparison, which would strengthen our findings, could not be performed.

During the observation time, R&M were recorded for each patient in the study group.

Of the 139 patients in group 2, 86 had postoperative R&M, and the relapse rate was 61.9%. Group 1 had a relapse rate of 41.2% (P = 0.002). The relapse rate of group 2 was much higher than that of group 1.

Reports about MIF allelomorphs are rare at present, and research on MIF-794CATT promoters is even rarer. Most studies have focused on inflammatory diseases, including autoimmune arthritis, asthma, psoriasis, ulcerative colitis, and others (De Benedetti et al., 2003; Donn et al., 2004; Nohara et al., 2004; Mizue et al., 2005; Wu et al., 2009). Given the results of our study, it is obvious that high-repetitive-sequence genotypes have a high positive correlation to malignancy grade. McDevitt et al. (2011) discovered that Saharan people have a higher frequency of MIF-794CATT promoter microsatellite repetitive sequences. The low-repetitive-sequence genotype is related to low morbidity of pernicious malaria complications, which suggests that MIF-794$CATT_5$ is a protective genotype against these complications. The $CATT_7$ repetitive sequence is related to high morbidity in prostate carcinoma, and patients with MIF-794$CATT_7$ have 5 times the risk of developing prostate carcinoma (Meyer-Siegler et al., 2007). Arisawa et al. (2008) reported that MIF-794$CATT_7$ increases the risk of gastric cancer, a finding consistent with those of the present study.

Our study examined 5 aspects - average survival time, TNM stage, differentiation grade, R&M, and survival rate - that inform HCC prognosis (Farinati et al., 2000; Seki et al., 2000). The results demonstrate that 1) the MIF-794$CATT_{5-8}$ promoter low-repetitive-sequence genotype (5/5+5/6+6/6) confers a better prognosis than that of the high-repetitive-sequence genotype (7/x+8/x), and 2) the MIF-794CATT promoter microsatellite repetitive sequence contributes to the postoperative prognosis of HCC. The deficiencies of the present study include the small number of HCC patients recruited, which excluded the possibility of performing statistical analysis and allowed the division of patients into only 2 groups. If the relationship between every genotype and HCC prognosis could be analyzed, the conclusion would be more valuable. Subsequent research will require a higher number of HCC patients and additional analyses.

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