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BRIEF ARTICLE

CpG island methylator phenotype in plasma is associated with hepatocellular carcinoma prognosis

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Abstract

AIM: To evaluate the clinical significance of CpG island methylator phenotype (CIMP) in plasma and its association with hepatocellular carcinoma (HCC) progress.

METHODS: CIMP status of 108 HCC patients was analyzed using a methylation marker panel in tumor tissues and plasma with methylation-specific polymerase chain reaction. Fifteen samples of non-neoplastic liver tissues and 60 of plasma from healthy persons were examined simultaneously. Examined genes included *APC, WIF-1, RUNX-3, DLC-1, SFRP-1, DKK* and *E-cad*.

RESULTS: The frequencies of high-level methylation in HCC tissue and plasma were at least 15% for the seven genes: *APC*, 48/108, 44.44% in tissue and 26/108, 24.07% in plasma; *WIF-1*, 53/108, 49.07% in tissue and 35/108, 32.41% in plasma; *RUNX-3*, 52/108, 48.14% in tissue and 42/108, 38.89% in plasma; *DLC-1*, 38/108, 35.18% in tissue and 23/108, 21.30% in plasma; *SFRP-1*, 40/108, 37.04% in tissue and 31/108, 28.7% in plasma; *DKK*, 39/108, 36.1% in tissue and 25/108, 23.14% in plasma; and *E-cad*, 37/108, 34.3% in tissue and 18/108, 16.67% in plasma. CIMP+ ($\geqslant$ 3 methylated genes) was detected in 68 (60.2%) tumor tissue samples and 62 (57.4%) plasma samples. CIMP was not detected in non-neoplastic liver tissues or plasma of healthy persons. CIMP status in tumor tissues differed significantly in gender, hepatitis B surface antigen, alpha-fetoprotein, and tumor-node-metastasis stage ($P < 0.05$). Similar results were obtained with plasma samples ($P < 0.05$). There was no difference in CIMP status in age, presence of hepatitis C virus antibody, cirrhosis, number of nodes, number of tumors, tumor size, or Edmondson-Steiner stage. A one-year follow-up found that the metastatic rate and recurrence rate in the CIMP+ group were significantly higher than in the CIMP- group as assessed with plasma samples ($P < 0.05$).

CONCLUSION: Plasma DNA can be a reliable sample source for CIMP analysis. CIMP in plasma may serve as a molecular marker of late-stage and poor-prognosis HCC.

Key words: CpG island methylator phenotype; Methylation; Plasma; Prognosis; Hepatocellular carcinoma

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INTRODUCTION

Hepatocellular carcinoma (HCC) is the fifth most common cancer in the world[1] and one of the leading causes of cancer-related death[2] because of late-stage diagnosis, lack of effective therapy, and easy recurrence[3]. Despite much progress in diagnostic and treatment strategies for HCC, recurrence and metastasis remain the main factors affecting patient survival. The 5-year survival rate is between 35% and 41% after resection[4,5] and between 47% and 61% after liver transplantation[6]. In general, prognosis remains poor, thus identification of useful molecular prognostic markers is necessary.

DNA methylation is an enzyme-mediated chemical modification that usually occurs in cytosine-guanine dinucleotide-rich areas (CpG islands) in the promoter region of genes. Aberrant promoter hypermethylation is an important mechanism leading to loss of gene function in tumors[7] including HCC[8,9]. The methylation pattern of multiple genes can provide useful information on global epigenetic alterations[10]. The hypermethylated subtype in tumors, called the CpG island methylator phenotype (CIMP) in which multiple genes are concurrently methylated, is a novel marker of tumor progression[11,12]. Methylation of CpG islands in the promoters of many tumor suppressor genes effectively silences those genes[13]. These epigenetic alterations may be important early events in carcinogenesis and may also be potential biomarkers for early detection[14]. CIMP is an important mechanism in HCC development and may serve as a molecular marker of late-stage HCC with poor prognosis[15]. Tumor tissue is the current gold standard to detect methylation in HCC[16]. However, not all patients' tissue samples can be used, but blood plasma samples are readily available from all patients.

Because determining the methylation status of single genes has limited prognostic value[17], CIMP has been reported as a promising predictive biomarker in many human cancers[17-19]. Many studies have investigated the relationship between CIMP and HCC prognosis[15,20], but these studies used tumor tissue as a research tool. These studies only examined the patients who underwent hepatic resection or liver puncture. Promoter methylation in breast cancer can be detected in the circulating plasma DNA[21]. The usefulness of future DNA methylation studies will be greatly enhanced if the efficacy is equivalent by using plasma and tumor tissues (Iyer *et al*[22], 2010). In this study, we analyzed the CIMP status of 108 HCC patients based on a methylation marker panel. Examined genes included *APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK* and *E-cad*. These genes were selected because they have been found to be frequently methylated in HCC and other malignancies. The aim of our current study was to evaluate the clinical significance of CIMP in plasma DNA and its association with prognosis in HCC.

MATERIALS AND METHODS

Patients and specimens

Between 2008 and 2010, 108 paired samples of primary HCC and corresponding non-neoplastic liver tissues and plasma from Chinese patients were collected during surgical resection at the Nantong Tumor Hospital. Samples from 60 healthy persons were used as matched controls. The controls had no self-reported history of cancer and were frequently matched to age (± 5 years), gender, and residential area. After resection, tissue specimens were immediately frozen in liquid nitrogen and stored at -70 ℃. Patients consisted of 85 men and 23 women, ranging in age from 32 to 74 (52.48 ± 7.56) years. Questionnaire data and blood samples were also collected from all patients and controls. After centrifugation, separated plasma samples were stored at -70 ℃. The diagnosis of HCC was confirmed by pathological examination or elevation of alpha-fetoprotein (AFP, > 400 g/L) combined with imaging examinations including magnetic resonance imaging (MRI) and/or computerized tomography (CT). Tumor-node-metastasis (TNM) stage was classified according to the 6th edition TNM classification of the American Joint Committee on Cancer (Greene *et al*, 2002). Fifteen samples of noncancerous liver tissues were obtained from patients with liver hemangioma. Eighty-six patients had cirrhosis, 84 patients were positive for hepatitis B surface antigen (HBsAg), and 19 patients were positive for hepatitis C virus antibody (anti-HCV). Written informed consent was obtained from each patient, and the study protocol was approved by the local ethics committee.

DNA extraction and sodium bisulfite

DNA from both plasma (200 μL per column) and tissue samples was treated with proteinase K and then extracted with phenol-chloroform according to the manufacturer's instructions (Shanghai ShineGene Molecular Biotech, Inc., Shanghai, China) and stored at -20 ℃. A final elution volume of 50 μL was collected. The extracted DNA was treated with sodium bisulfite using an EZ DNA-methylation™ kit (Zymo Research, Orange, CA, United States) to convert all unmethylated cytosines to uracils. Bisulfite-modified DNA was resuspended in 10 μL elution buffer and stored at -20 ℃ until methylation-specific polymerase chain reaction (MSP).

MSP

MSP was used to determine the methylation status of CpG islands in genes after bisulfate treatment[23,24]. The methylation status of the promoters of *APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK* and *E-cad* was determined with a two-step MSP protocol that consists of amplification and detection. Polymerase chain reaction (PCR) products were analyzed on 2% agarose gels, stained with ethidium bromide, and visualized with ultraviolet illumina-

Table 1 Primer sets for nested methylation-specific polymerase chain reaction

Genes			Primer sequence (5'-3')
APC	U	F	GTGTTTTATTGTGGAGTGTGGGTT
		R	CCAATCAACAAACTCCCAACAA
	M	F	TATTGCGGAGTGCGGGTC
		R	TCGACGAACTCCCGACGA
WIF-1	U	F	GGGTGTTTTATTGGGTGTATTGT
		R	AAAAAAACTAACACAAACAAAATACAAAC
	M	F	CGTTTTATTGGGCGTATCGT
		R	ACTAACGCGAACGAAATACGA
RUNX3	U	F	TTATGAGGGGTGGTTGTATGTGGG
		R	AAAACAACCAACACAAACACCTCC
	M	F	TTACGAGGGGCGGTCGTACGCGGG
		R	AAAACGACCGACGCGAACGCCTCC
DLC-1	U	F	AAACCCAACAAAAAAACCCAACTAACA
		R	TTTTTTAAAGATTGAAATGAGGGAGTG
	M	F	CCCAACGAAAAAACCCGACTAACG
		R	TTTAAAGATCGAAACGAGCGAGCG
SFRP-1	U	F	GAGTTAGTGTTGTGTGTTTGTTGTTTTGT
		R	CCCAACATTACCCAACTCCACAACCA
	M	F	GTGTCGCGCGTTCGTCGTTTCGC
		R	AACGTTACCCGACTCCGCGACCG
DKK	U	F	TTAGGGGTGGGTGGTGGGGT
		R	CTACATCTCCACTCTACACCCA
	M	F	GGGGCGGGCGGCGGGGC
		R	ACATCTCCGCTCTACGCCCG
E-cad	U	F	TAATTTTAGGTTAGAGGGTTATTGT
		R	CCACCCCAATACTAAATCACAACA
	M	F	TTAGGTTAGAGGGTTATCGCGT
		R	TAACTAAAAATTCACCTACCGAC

M: Methylated sequence; U: Unmethylated sequence; F: Forward sequence; R: Reverse sequence.

tion. All experiments were performed in duplicate. Table 1 lists the primer sequences.

Follow-up

Patients were followed up for one year. The last follow-up was on September 8, 2010. None of the patients received chemotherapy prior to surgery. The median follow-up time was 5 mo (range, 3-12 mo). Patients were given a physical examination, abdominal ultrasonography, and chest X-ray, and serum was collected and tested for AFP. During the first year, local recurrence and distant metastasis were monitored every 3 mo with CT and/or MRI. Only 98 of the 108 patients were completely followed up. Twenty-one patients (19.44%) were found to have HCC recurrence; these patients all had AFP levels >20 μg/L, and CT examination revealed a clear image of the liver cancer. These 21 patients appeared to have different degrees of multicentric new lesions of the liver, including multiple nodules, apparent mass, the portal vein, hepatic vein thrombosis and sub-lesions. Twenty-four (22.2%) patients had HCC metastasis. According to their clinicopathological characteristics, formation of portal vein tumor thromboses, and dissemination into lymph nodes, 18 patients showed liver metastasis, four patients had bone metastasis, and two patients had brain metastasis. Forty-four (40.74%) patients died of cancer-related causes. Fifty-four patients were still alive at the time of the last follow-up.

Table 2 Frequencies of gene methylation in hepatocellular carcinomas, corresponding non-neoplastic liver tissues and normal liver tissues

Genes	No. of methylated samples					
	Tumor (n = 108)	Plasma (n = 108)	Non-neoplastic tissues (n = 102)	Normal tissues (n = 15)	Healthy plasma (n = 60)	χ^2
APC	48[1]	26	5	0	0	43.47
WIF- 1	53[1]	35	6	0	0	48.44
DLC-1	38[1]	23	4	0	0	32.05
DKK	39[1]	25	5	0	0	30.85
SFRP-1	48[1]	26	5	0	0	43.47
E-cad-1	37[1]	18	3	0	0	33.37
RUNX3	52[1]	42	4	0	0	52.47

[1]χ^2 test comparing the frequencies of gene methylation between tumor and non-neoplastic samples, $P < 0.05$.

Statistical analysis

The SPSS 13.0 software package (SPSS, Inc., Chicago, IL, United States) was used for all statistical analyses. Values for the clinical and biological characteristics of patients were expressed as means ± SD. Comparison was done with Student's t test. A χ^2 test or Fisher's exact test was used to compare the incidence of methylation. All P values presented are two-sided, and a P value of less than 0.05 was considered statistically significant.

RESULTS

Clinical data

Median tumor size was 6.0 cm (range, 1.2-19.4 cm). According to the Edmondson-Steiner classification system, 7 cases were classified as grade Ⅰ, 55 cases were grade Ⅱ, 43 cases were grade Ⅲ, and 3 cases were grade Ⅳ. According to the 6th edition TNM classification of the American Joint Committee on Cancer (Greene *et al*, 2002), 51 cases were classified as grade Ⅰ, 20 cases were grade Ⅱ and 37 cases were grade Ⅲ.

Methylation of tumor-associated genes in HCC

We examined the methylation status of seven tumor-associated genes (*APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK*, *E-cad*) in tissue and plasma samples from HCC patients and controls. The results are summarized in Table 2. No methylation was detected in the promoters of these genes in normal tissues or control plasma. Table 2 shows that methylation was more frequent in HCC than in adjacent non-neoplastic liver tissue and normal liver tissues for all seven genes. The same results were observed for plasma (Table 2). Methylation at any level was detected in one or more of the genes in 99 (91.67%) of 108 cases. The frequencies of high-level methylation in HCC tissue and plasma were at least 15% for the seven genes, including 44.44% for *APC*, 49.07% for *WIF-1*, 48.14% for *RUNX3*, 35.18% for *DLC-1*, 37.04% for *SFRP-1*, 36.1% for *DKK*, and 34.3% for *E-cad* in tissue. In plas-

Table 3 Relationship between CpG island methylator phenotype and clinicopathological characteristics in hepatocellular carcinomas

Characteristics	CIMP+		CIMP-		χ^{21}	χ^{22}
	Tumor	Plasma	Tumor	Plasma		
Age (yr)						
< 52	27	25	21	23	0.5584	1.002
⩾ 52	38	37	22	23		
Gender						
Female	9[a]	8[a]	13	15	4.28	6.115
Male	56	54	30	31		
HBsAg						
Negative	9[a]	7[a]	15	17	6.625	10.06
Positive	56	55	28	29		
Anti-HCV						
Negative	52	52	37	37	0.655	0.2151
Positive	13	10	6	9		
AFP (μg/L)						
< 20	2[a]	3[a]	40	41	88.1	77.72
20-400	29	30	3	5		
> 400	34	29	0	0		
Cirrhosis						
Yes	53	50	32	36	0.7827	0.0925
No	12	12	11	10		
Node number						
Single	44	37	31	32	0.2362	1.119
Multiple	21	25	12	14		
Tumor number						
Single	41	37	28	31	0.0467	0.6738
Multiple	24	25	15	15		
Tumor size						
< 6	30	27	24	22	0.966	0.195
⩾ 6	35	35	19	24		
Edmondson-Steiner						
Ⅰ-Ⅱ	38	35	24	21	0.0742	1.234
Ⅲ-Ⅳ	27	27	19	25		
TNM stage						
Ⅰ	31[a]	34[a]	20	17	5.325	4.872
Ⅱ	16	12	4	8		
Ⅲ	18	16	19	21		

[1]Indicates χ^2 test comparing the clinicopathological characteristics between CIMP+ and CIMP- in tumor tissues; [2]Indicates the comparison in the plasma. [a]$P < 0.05$. CIMP: CpG island methylator phenotype; HBsAg: Hepatitis B surface antigen; Anti-HCV: Hepatitis C virus antibody; AFP: Alpha-fetoprotein; TNM: Tumor-node-metastasis.

ma, the frequencies were 24.07% for *APC*, 32.41% for *WIF-1*, 38.89% for *RUNX3*, 21.30% for *DLC-1*, 28.7% for *SFRP-1*, 23.14% for *DKK* and 16.67% for *E-cad*.

Concordance of data obtained from plasma and tissue samples

The frequencies of promoter methylation in tissue and plasma samples for the seven tumor-associated genes were as follows: *APC*, 48/108, 44.44% in tissue and 26/108, 24.07% in plasma; *WIF-1*, 53/108, 49.07% in tissue and 35/108, 32.41% in plasma; *RUNX3*, 52/108, 48.14% in tissue and 42/108, 38.89% in plasma; *DLC-1*, 38/108, 35.18% in tissue and 23/108, 21.30% in plasma; *SFRP-1*, 40/108, 37.04% in tissue and 31/108, 28.7% in plasma; *DKK*, 39/108, 36.1% in tissue and 25/108, 23.14% in plasma; and *E-cad*, 37/108, 34.3% in tissue and 18/108, 16.67% in plasma. We observed significant concordance of promoter methylation between plasma and tissue samples for all seven genes.

CIMP in HCC

According to the criteria in a related study[25], the CIMP status of each of our 108 HCC samples was classified as CIMP+ (with ⩾ 3 methylated genes) or CIMP- (with two or fewer methylated genes). In this study, because of a cutoff value of 3, the average number of methylated genes was 2.8 in tumor tissue and 2.0 in plasma. Regarding tumor tissues, 65 cases of HCC (60.2%) were classified as CIMP+, and 43 (39.8%) cases were classified as CIMP-. When plasma was examined, 62 cases of HCC (57.4%) were classified as CIMP+, and 46 cases (42.6%) were classified as CIMP-. No CIMP+ samples were from non-neoplastic tissues or healthy controls.

Relationship between CIMP and clinicopathological characteristics

We analyzed the relationship between CIMP and clinicopathological characteristics including age, gender, HBsAg, anti-HCV, serum AFP level, liver cirrhosis, number of nodes, tumor size, number of tumors, Edmondson-Steiner grading, and TNM stage. In tumor tissues, we found significant differences between CIMP and gender, HBsAg, AFP, and TNM stage ($P < 0.05$ for all; Table 3). The same result was obtained with plasma samples, including differences between CIMP and gender, HBsAg, AFP, and TNM stage ($P < 0.05$ for all; Table 3). There were no differences between CIMP status and age, anti-HCV, cirrhosis, number of nodes, number of tumors, tumor size, or Edmondson-Steiner stage.

Prognostic significance of CIMP in HCC

Follow-up for up to one year found that the rates of metastasis differed significantly between the CIMP+ and CIMP- groups when tumor tissue ($P < 0.05$) and plasma were examined ($P < 0.05$). CIMP+ tumors appeared to frequently undergo metastasis. The recurrence rates were significantly higher in the CIMP+ group compared with the CIMP- group when both tumor tissue and plasma were examined ($P < 0.05$ for both). Survival rates differed significantly between the CIMP+ and CIMP- groups ($P < 0.05$ for both; Table 4).

DISCUSSION

Little is known about the many risk factors that are likely to affect the metastasis and recurrence of HCC. The development and progression of HCC is a multistep process, and the basic molecular pathway of HCC development remains largely unknown. Abnormal gene expression may be an early event in tumorigenesis and a potential biomarker for early detection[14]. Recurrence, which is frequent after surgical resection, and metastasis are the main factors affecting the long-term prognosis of HCC patients. Currently, predicting the development and guiding the treatment for HCC are generally based on clinical characteristics and/or the stage of HCC[26].

Table 4 Relationship between CpG island methylator phenotype and prognosis of hepatocellular carcinomas after a one-year follow-up ($n = 98$)

Prognosis	CIMP+		CIMP-		χ^{21}	χ^{22}
	Tumor	Plasma	Tumor	Plasma		
Metastasis						
Yes	22[a]	19[a]	2	5	11.032	5.762
No	40	38	34	36		
Recurrence						
Yes	18[a]	17[a]	3	4	5.796	4.257
No	44	40	33	37		
Survival						
Yes	26[a]	23[a]	24	27	5.574	4.621
No	36	32	12	16		

[1]Indicates χ^2 test comparing the prognosis between CIMP+ and CIMP- in tumor tissues; [2]Indicates the comparison in the plasma. [a]$P < 0.05$. CIMP: CpG island methylator phenotype.

However, genetic and epigenetic changes can also be used as indicators of cancer progression and/or markers. Epigenetic processes, in particular, abnormal methylation of CpG islands in HCC, may play a crucial role in cancer development[27,28]. In our study, we examined the methylation status of seven tumor-associated genes (*APC*, *WIF-1*, *RUNX-3*, *DLC-1*, *SFRP-1*, *DKK* and *E-cad*) on the basis of their biological significance in tissue and plasma in HCC and controls. We observed no methylation in the promoters of these genes in normal tissues and control plasma. The frequencies of high-level methylation in HCC tissue and plasma were at least 15% for these seven genes. The results showed that examining the methylation status of multiple genes may aid the diagnosis of early HCC. In this study, we also found that promoter methylation in plasma DNA was highly specific for certain cancer-related genes and that results for plasma were similar to those for tissue samples. There was good concordance in DNA methylation between plasma and tumor tissues in HCC.

CIMP+ tumors tend to occur in older patients with colorectal cancer, and this phenotype is overrepresented in proximal colon cancer tumors[29]. In esophageal adenocarcinoma, CIMP is associated with poor prognosis[30]. CIMP is also associated with environmental factors and serum AFP levels in HCC[31,32]. Many studies have investigated the relationship between CIMP and HCC prognosis[15,20]. However, these studies used tumor tissue samples, thereby limiting the applicability of the research. In our study, we found that CIMP in tumor tissues as well as plasma was associated with metastasis and recurrence of HCC. The presence of CIMP+ tumors indicates the likelihood of metastasis and recurrence in HCC. Thus, CIMP may play an important role in the pathogenesis, metastasis, and recurrence of HCC. Methylation may silence the genes associated with suppression of metastasis and recurrence. We also observed that there were no CIMP+ samples from corresponding non-neoplastic tissues and healthy controls, consistent with the known importance of methylation in cancer development. Our study also shows that promoter methylation in plasma DNA was highly specific and was seen in both plasma and tissue samples. Therefore, CIMP detection in plasma rather than tumor tissues may be used as a reliable source for predicting the prognosis of patients with HCC.

It has been shown that methylation of genes associated with tumors and CIMP is intimately involved in the early process of carcinogenesis and tumor progression[13]. We found that CIMP is a common phenomenon in HCC. Thus, CIMP may ultimately offer a new tool for predicting patients' clinical outcomes.

CIMP that is associated with tumors seems to have distinct epidemiology, histology, and molecular features[20].

The functional significance of methylation of tumor-associated genes in HCC may be that this process initiates progressive inactivation of these genes[15]. CIMP may be useful for stratifying the prognosis of patients with TNM stage I HCC and for identifying patients who are at higher risk for recurrence[20]. In our study, the presence of CIMP+ tumors indicates a poor prognosis in HCC. Thus, CIMP detection may determine the prognosis of patients with HCC. Furthermore, our study shows that promoter methylation in plasma DNA was highly specific and plasma and tissue samples yielded similar results. Therefore, CIMP detection in plasma, rather than tumor tissues, may be used as a reliable index for predicting the course of patients with HCC. Limitations of this study are that the results were based on a relatively small sample of patients, and the number of cancer-related genes we examined was small. Furthermore, the length of follow-up was only one year. Thus, in the future, a larger-scale multi-gene study that includes an extended follow-up period is needed to confirm our results.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the the fifth most common malignant cancer in the world, for later diagnosis, lack of effient therapy and easy recurrence. Despite a lot of progress in diagnostic and treatment strategies for HCC, recurrence and metastasis are still the main factors affecting the long-term prognosis for patients. In general, prognosis is still poor, and identification of useful molecular prognostic markers is required.

Research frontiers

DNA methylation is an enzyme-induced chemical modificationthat usually occurs in cytosine-guanine dinucleotide-rich areas (CpG islands) in the gene promoter regions. Aberrant promoter hypermethylation is an important mechanism for loss of gene function in tumors including HCC. The methylation pattern of multiple genes can provide useful information and an overall picture of epigenetic alterations. The hypermethylated subtype in tumors, called the CpG island methylator phenotype (CIMP), where multiplegenes are concurrently methylated, is a novel marker for tumor for progression. CIMP is an important mechanism in hepatocellular carcinoma development and could serve as a molecular marker of late stage and poorly prognostic HCC development.

Innovations and breakthroughs

Many studies have investigated the relationship between CIMP and prognosis associated with HCC, and also made many admirable achievements. However, these studies based on tumor tissue as a research object. These patients were only confined to patients with hepatic resection or liver puncture and part of the patients would be missed. Plasma DNA can be reliable for testing methylation profile in hepatocellular carcinoma patients. The productivity level of future DNA methylation studies will be greatly enhanced if the efficacy of using plasma is

equivalent to tumor tissue.In this study, the authors analysed the CIMP status of HCC patients based on a methylation marker panel. Associated genes include the *APC*, *WIF-1*, *RUNX3*, *DLC-1*, *SFRP-1*, *DAPK* and *E-cad*. These genes were selected because they have been found to be methylated frequently in HCC and other malignancies. The aim of the present study was to evaluate the clinical significance of CIMP in plasma associated with prognosis in HCC.

Applications

In this study, the authors found that plasma DNA could be used as a reliable resource and replace tumor tissue for CIMP research. This results also suggested that CIMP in plasma could serve as a molecular marker of late stage and poorly prognostic HCC.

Terminology

Epigenetic changes: Heritable changes in gene structure that without changing the gene sequence. CpG islands: CpG rich areas located in the promoter regions of many genes. CpG island methylation: The addition of a methyl group to a cytosine residue that lies next to guanine within CpG dinucleotides. CIMP: The hypermethylated subtype in tumors, called the CIMP, where multiplegenes are concurrently methylated.

Peer review

This article is new and clinically informative in that it has firstly reported that the CIMP in plasma is associated with the presence of HCC.

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