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World J Gastroenterol 2007 August 28; 13(32): 4345-4349
World Journal of Gastroenterology ISSN 1007-9327

RAPID COMMUNICATION

Role of serum interleukin-18 as a prognostic factor in patients with hepatocellular carcinoma

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Received: 2007-02-12 Accepted: 2007-03-15

Abstract

AIM: To determine whether serum interleukin-18 (IL-18) levels correlated with clinicopathologic features and prognosis in patients with hepatocellular carcinoma (HCC).

METHODS: Serum IL-18, IL-6 and IL-12 levels were measured by enzyme-linked immunosorbent assay (ELISA) from 70 patients with HCC and 10 healthy controls.

RESULTS: Serum IL-18, IL-6 and IL-12 levels of patients with HCC were significantly higher that those of the controls. The levels of IL-18 correlated significantly with the presence of venous invasion and advanced tumor stages classified by Okuda's criteria. Patients with high serum IL-18 levels ($\geqslant 10^5$ pg/mL) had a poorer survival than those with low serum IL-18 levels ($< 10^5$ pg/mL) (4 and 11 mo, respectively, $P = 0.015$). Multivariate analyses showed that serum IL-18 level, but not IL-6 and IL-12 levels, was a significant and independent prognostic factor of survival.

CONCLUSION: These findings demonstrate that serum IL-8 may a useful biological marker of tumor invasiveness and an independent prognostic factor of survival for patients with HCC. Thus, the detailed mechanisms of IL-18 involving in tumor progression should be further investigated.

Key words: Hepatocellular carcinoma; Interleukin-18; Serum marker; Prognosis

Tangkijvanich P, Thong-ngam D, Mahachai V, Theamboonlers A, Poovorawan Y. Role of serum interleukin-18 as a prognostic factor in patients with hepatocellular carcinoma. *World J Gastroenterol* 2007; 13(32): 4345-4349

http://www.wjgnet.com/1007-9327/13/4345.asp

INTRODUCTION

Hepatocellular carcinoma (HCC) represents one of the most common cancers worldwide with a particularly high prevalence in sub-Saharan Africa and Southeast Asia where hepatitis B virus (HBV) and hepatitis C virus (HCV) infections are common[1]. Although recent advances in the detection and treatment of HCC have improved the survival, the prognosis of most patients is somewhat unsatisfactory due to rapid clinical deterioration after the initial diagnosis and high incidence of recurrence after surgical resection[2]. In general, the natural history of HCC depends on the severity of the underlying liver disease, tumor characteristics and the efficacy of treatment interventions[3]. Besides these features, a number of biological markers including cytokines and growth factors have been demonstrated to be increased in the sera of patients with HCC and may be associated with a poor prognosis.

Interleukin-18 (IL-18), originally known as interferon-γ (IFN-γ)-inducing factor (IGIF), is a cytokine that shares structural and functional properties with interleukin-1 (IL-1)[4,5]. This cytokine is mainly produced by activated macrophages, but may also be expressed by Kupffer cells, T cells, B cells, keratinocytes, astrocytes, and osteoblasts[6]. Like IL-1, IL-18 is synthesized as an inactive precursor (pro-IL-18, 24 kDa), which is cleaved by interleukin-1 β-converting enzyme (ICE or caspase-1) into an active 18 kDa mature form[6-8]. IL-18 has multiple biological activities via its capacity to stimulate innate immunity and both Th1 and Th2 mediated responses[6,8]. It also exerts anti-tumor effects that are mediated by enhancement of NK cell activity, reduction of tumorigenesis, induction of apoptosis and inhibition of angiogenesis in tumor cells[9,10]. In addition, recent data have been suggested that inappropriate production of

IL-18 contributes to the pathogenesis of cancers and may influence the clinical outcome of patients[11]. Specifically, it has been demonstrated that serum IL-18 level may have prognostic significance in some types of cancer including colonic carcinoma, gastric carcinoma, esophageal carcinoma, breast cancer, and hematologic malignancies[12-16]. However, the prognostic role of serum IL-18 level in patients with HCC has never been investigated. Therefore, in this study, we determined whether serum IL-18 level correlated with clinicopathologic features and prognosis of patients with HCC.

MATERIALS AND METHODS

Patients and blood samples

For the purpose of this study, 70 patients with HCC were randomly selected from a pool of patients with chronic liver disease who were seen and followed at King Chulalongkorn Memorial Hospital (Bangkok, Thailand) between August 1997 and September 2003. The control group comprised 10 healthy adults from the blood bank. Serum samples were collected from each subject at the time of their clinical evaluation and stored at -70℃ until further tested. The study was approved by the Ethical Committee of the Faculty of Medicine, Chulalongkorn University. Informed consent was obtained according to the regulations of the committee.

The diagnosis of HCC was based on histopathology and/or a combination of mass lesions in the liver on hepatic imaging and serum alpha-fetoprotein (AFP) levels above 400 ng/mL. All demographic and clinical data were extracted from patients' files. The authors collected the data including sex and age, as well as clinical data such as liver function tests, severity of liver disease graded as the Child-Pugh status, Okuda staging, etiologic factors (HBsAg, Anti-HCV or alcohol abuse), serum AFP levels at the time of diagnosis, and presence of venous invasion diagnosed by CT scan.

Hepatitis B surface antigen (HBsAg), hepatitis C virus antibody (anti-HCV) and AFP level were determined by enzyme-linked immunosorbent assays (ELISA) using commercially available kits (Auszyme Ⅱ, Abbott Laboratories, IL for HBsAg; ELISA Ⅱ, Ortho Diagnostic Systems, Chiron Corp., CA for anti-HCV; and Cobus® Core, Roche Diagnostics, Basel, Switzerland for AFP). Biochemical liver function tests were determined by automated chemical analyzer (Hitachi 911) at the central laboratory of the hospital.

Measurement of serum IL-6, IL-12 and IL-18 levels

Serum IL-6, IL-12 and IL-18 were determined by using ELISA kits (R&D systems, Inc., Minneapolis, MN). ELISA was performed according to the manufacturer' instructions.

Statistical analysis

Data are expressed as percentage, mean and standard deviation. Comparisons between groups were analyzed by the χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney test or Student's t test when appropriate for quantitative variables. Survival curves were constructed using the Kaplan-Meier method and difference between curves was testing by the log-rank test. The Cox regression analysis was performed to identify which independent factors have a significant influence on the overall survival. *P* values below 0.05 for a two-tailed test were considered statistically significant. All statistical analyses were performed using the SPSS software for windows 10.0 (SPSS Inc., Chicago, IL).

Table 1 Clinical and demographic data of patients with HCC at the time of the diagnosis

Clinical features	mean ± SD or percentage (%)
Age (yr)	55.0 ± 13.6 (range, 26-89)
Sex (male:female)	60:10
Etiology	
Alcohol dependence	9/70 (12.8)
HBsAg-positive	39/70 (55.7)
Anti-HCV-positive	7/70 (10)
HBsAg- and anti-HCV-positive	2/70 (2.9)
Unknown	13/70 (18.6)
Liver function test	
Total bilirubin (mg%)	2.3 ± 3.5
Albumin (g/dL)	3.5 ± 0.7
AST (IU/L)	150.9 ± 130.4
ALT (IU/L)	82.9 ± 84.1
Alkaline phosphatase (IU/L)	512.0 ± 317.9
Prothrombin time (s)	14.4 ± 4.2
Child-Pugh classification	
A	43/70 (61.4)
B	23/70 (32.9)
C	4/70 (5.7)
Okuda staging system	
1	19/70 (27.2)
2	46/70 (65.7)
3	5/70 (7.1)
Venous invasion	17/70 (24.3)
Extrahepatic metastasis	12/70 (17.1)
AFP (≥ 400 ng/mL)	27/70 (38.6)

RESULTS

The clinical data of patients with HCC

The clinical and demographic data of patients with HCC in this study are shown in Table 1. Among the 70 recruited patients, 60 were men and 10 were women. The average age of the patients was 55.0 ± 13.6 years (ranged 26-89 years). All patients had underlying cirrhosis. Seventeen patients (24.3%) had venous invasion. Extrahepatic metastasis was found in 12 patients (17.1%). The mean total bilirubin (TB), serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT), albumin, alkaline phosphatase (AP), and prothrombin time (PT) were 2.3 ± 3.4 mg/dL, 150.9 ± 130.4 IU/L, 82.9 ± 84.1 IU/L, 3.5 ± 0.7 g/dL, 512.6 ± 317.9 IU/L, and 14.4 ± 4.2 sec, respectively. Twenty-seven patients (38.6%) had serum AFP higher than 400 ng/mL. According to Okuda staging system, there were 19 patients (27.2%) in stage 1, 46 patients (65.7%) in stage 2, and 5 patients (7.1%) in stage 3.

For predisposing etiologic factors, 9 patients (12.8%) were associated with alcohol-dependent. Thirty-nine patients (55.7%) were associated with HBsAg-positive, and 7 patients (10%) were associated with anti-HCV-positive.

Table 2 Serum levels of interleukins in patients with HCC and in healthy controls

	Healthy controls (n = 10)	HCC patients (n = 70)	P
IL-6 (pg/mL)	2.9 ± 13.4	31.2 ± 52.2	0.01
IL-12 (pg/mL)	1.5 ± 0.9	6.2 ± 9.6	0.03
IL-18 (pg/mL)	38.5 ± 22.4	104.6 ± 65.8	0.002

Two patients (2.9%) had both HBsAg-positive and anti-HCV-positive. The predisposing factors could not be determined in 13 patients (18.6%).

Seven patients (10%) had undergone surgical resection, 19 patients (27.1%) had been treated with transarterial chemoembolization (TACE), and the remaining 44 patients (62.9%) had received no specific treatment because of their advanced tumor stage or refusal to therapy.

Serum IL-18 levels of patients with HCC and the survival

As shown in Table 2, serum IL-18 levels in patients with HCC were significantly elevated compared with those of the controls (104.6 ± 65.8 *vs* 38.5 ± 22.4 pg/mL, $P = 0.002$). Similarly, the levels of serum IL-6 and IL-12 in patients with HCC were significantly increased compared with healthy subjects (31.2 ± 52.2 *vs* 2.9 ± 13.4 pg/mL, $P = 0.01$, and 6.2 ± 9.6 *vs* 1.5±0.9 pg/mL, $P = 0.02$, respectively). Serum IL-18 levels also exhibited a positive correlation with serum IL-6 and IL-12 levels ($P = 0.021$; Pearson $r = 0.276$ and $P = 0.002$; Pearson $r = 0.369$, respectively).

In order to evaluate the association between serum IL-18 and the survival, the patients with HCC were further categorized into two groups according to their serum IL-18 levels. In this respect, the cut point of 10^5 pg/mL, which represented the mean serum IL-18 level in the whole group, was used. There were 41 patients with serum IL-18 < 10^5 pg/mL and 29 patients with serum IL-18 ≥ 10^5 pg/mL. There was no statistically significant difference in age, gender, serum ALT, AST, AFP, PT and extrahepatic metastasis between these two groups (Table 3). However, patients with high serum IL-18 levels had significantly lower mean serum albumin level ($P = 0.01$), but had significantly higher mean total bilirubin ($P = 0.03$), serum AP levels ($P = 0.04$), exhibited more advanced tumor stages classified by Okuda's criteria ($P = 0.03$), and had higher percentage of venous invasion ($P = 0.02$) than patients with low serum IL-18 levels.

Kaplan-Meier survival curves revealed that the median survival of patients with low serum IL-18 and the other were 10.5 and 5.0 mo, respectively (Figure 1A). By using log-rank test, there was a statistically significant difference in the median survival between these two groups ($P = 0.007$). Among patients who were treated with surgery or TACE, the medial overall survival for the low and high serum IL-18 groups were 18.5 and 10.0 mo, respectively ($P = 0.021$) (Figure 1B). In untreated cases, the medial overall survival for the low and high serum IL-18 groups were 4.5 and 2.7 mo, respectively ($P = 0.043$) (Figure 1C).

Serum IL-18, IL-6 and IL-12 levels were entered into a Cox regression analysis together with other variables that may influence prognosis. These included age, gender, serum AFP level, HBsAg status, tumor size, tumor number, venous invasion, extrahepatic metastasis, Child-Pugh classification, Okuda staging, and therapy of HCC. Multivariate analyses revealed that independent prognostic factors of overall survival included high serum IL-18 level, venous invasion and no receiving therapy for HCC (Table 4).

Table 3 Comparison of clinical data of patients with HCC according to serum IL-18 levels

Clinical features	IL-18 < 10^5 pg/mL (n = 41)	IL-18 ≥ 10^5 pg/mL (n = 29)	P
Age (yr)	54.9 ± 15.3	55.2 ± 11.1	NS
Sex (male:female)	34:7	26:3	NS
Liver function test			
Total bilirubin (mg%)	1.4 ± 0.7	3.6 ± 4.9	0.03
Albumin (g/dL)	3.8 ± 0.7	3.3 ± 0.7	0.01
AST (IU/L)	136.8 ± 116.4	170.8 ± 148.3	NS
ALT (IU/L)	66.5 ± 39.2	106.2 ± 119.7	NS
Alkaline phosphatase (IU/L)	436.9 ± 275.2	612.1 ± 348.2	0.04
Prothrombin time (s)	13.9 ± 2.4	14.9 ± 5.8	NS
Okuda staging (1:2:3)	15:25:1	4:21:4	0.03
Venous invasion (+:-)	6:35	11:18	0.02
Extrahepatic metastasis (+:-)	6:35	6:29	NS
AFP (< 400 ng/mL: ≥ 400 ng/mL)	25:16	18:11	NS

DISCUSSION

Enhanced expression of proinflammatory, hematopoietic and angiogenic cytokines has been demonstrated in several human tumors[17]. Some of these cytokines may act as autocrine or paracrine tumor cell growth factors, inhibitors of apoptosis, attractors of immune cells, and promoters of angiogenesis[18,19]. Accordingly, it is likely that the deregulation of these cytokines may contribute to the development or progression of the malignant process. Currently, serum levels of several cytokines have been found to be increased in patients with HCC and may be correlated with clinical outcomes. For instance, higher level IL-10 was observed in patients with HCC[20], and increased IL-10 values were associated with a poor prognosis in patients undergoing surgical resection[20], as well as in patients with unresectable tumor[21] Similarly, serum IL-8 was shown to be a useful biological marker of tumor invasiveness and an independent prognostic factor of survival for patients with HCC[22].

To the best of our knowledge, this is the first study demonstrating that the levels of serum IL-18 were markedly elevated in patients with HCC compared with healthy controls. In addition, our data showed that a high-serum IL-18 level was significantly correlated with advanced tumor stage classified by Okuda's criteria. Furthermore, serum IL-18 levels were significantly correlated with venous invasion, a pathobiological feature indicative of tumor aggressiveness. These data suggest that serum IL-18 might be useful in the clinical setting to predict tumor invasiveness and stage. A high-serum IL-18 level was also a significant prognostic factor in terms of overall survival, as demonstrated by multivariate analysis. These results were in agreement with previous data suggesting that serum IL-18 levels are related to the prognosis of patients with various

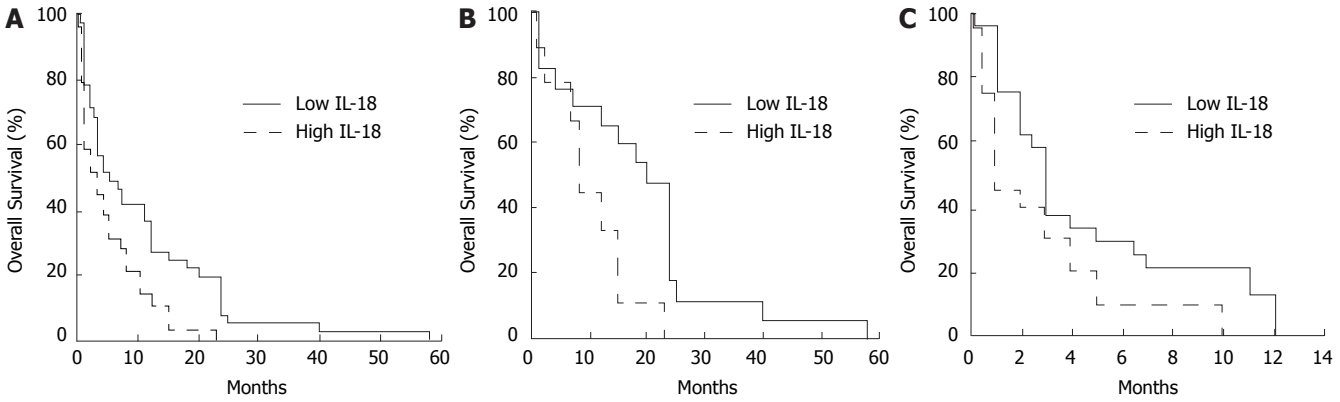

Figure 1 Overall survival of HCC patients with low serum IL-18 level (< 10^5 pg/mL) or high IL-18 level (≥ 10^5 pg/mL). **A**: All patients; **B**: Patients who were treated with surgery or TOCE; **C**: Untreated patients.

Table 4 Multivariate analysis of prognostic factors of survival with Cox's proportional hazards model

Factors	Risk ratio (95% CI)	*P*
High serum IL-18 level	1.86 (1.11-3.11)	0.019
Presence of venous invasion	2.09 (1.19-3.67)	0.010
No receiving therapy	5.01 (2.49-10.06)	< 0.001

malignant diseases in the gastrointestinal tract, including colonic, gastric and esophageal carcinoma[12,13,16]. In agreement with our results, it has been shown recently that the expression of IL-18 receptor in tumor tissues was found to be a significant predictor of a poor outcome in HCV-associated HCC patients[23].

The precise mechanisms underlying the positive correlation between serum IL-18 levels and advanced tumor stages are unclear. As previously mentioned, IL-18 exerts anti-tumor activity *via* several mechanisms including enhancement of NK cell function, induction of apoptosis *via* Fas/Fas ligand interaction and inhibition of angiogenesis[9,10]. Indeed, recent data have demonstrated that positive IL-18 immunoreactivity is significantly higher in the surrounding hepatocytes compared with the tumor portion from the same individual[24]. It has been shown that decreasing IL-18 production in tumor cells may be related to the down regulation of ICE gene expression, as demonstrated in colon and ovarian carcinoma[13,25]. In contrast, IL-18 and ICE transcripts have been detected in the corresponding normal colon and ovarian epithelium suggesting the bioactive IL-18 is most likely produced by the adjacent normal cells[13,25]. Taken together, it is speculated that IL-18 production by the normal adjacent hepatocytes may reflect the degree of defense mechanisms against tumor growth and dissemination of HCC.

IL-12, also known as NK cell stimulatory factor or cytotoxic lymphocyte maturation factor, is a multifunctional cytokine produced primarily by antigen-presenting cells (APC), such as monocytes and NK cells[26]. This cytokine augments proliferation, cytokine production and the development of Th1. Furthermore, IL-12, in combination with IL-18, induces anti-tumor effects against a variety of tumor cells via the activity of IFN-γ[27,28]. It has been shown that serum IL-12 levels are significantly higher in patients with gastric and esophageal cancers compared with healthy controls[12,29]. In patients with esophageal carcinoma, increasing serum IL-12 and IL-18 levels correlate with tumor growth and progression[12]. In contrast, serum IL-12 levels in patients with far-advanced gastric cancer are significantly lower that those with less-advanced stages[29]. In this study, we demonstrated that serum IL-12 levels were significantly higher in patients with HCC than in healthy controls. Furthermore, we found that its levels were correlation with IL-18 levels, suggesting that these cytokines may act synergistically in the anti-tumor activity. However, unlike IL-18, IL-12 levels were not confirmed as a prognostic factor in multivariate analysis.

IL-6 is a pleiotropic cytokine that was originally identified as a T cell-derived lymphokine inducing final maturation of B cells into antibody-producing cells[30]. This cytokine plays an important role in hematopoiesis, acute-phase responses and host defense mechanisms[31]. In addition, IL-6 has also shown to act as an autocrine growth factor in malignancy[30]. Increased serum levels of IL-6 have been demonstrated in patients with a variety of cancers and may be associated with a poor outcome[32-34]. However, the clinical significance of serum IL-6 levels in patients with HCC remains to be established[20,35,36]. In this study, we found that though levels of IL-6 were significantly higher in patients with HCC than in healthy subjects, its levels were not an independent prognostic factor in multivariate analysis. Thus, our results were in agreement with the reports conducted by Chau *et al* and Parasole *et al*[20,36].

In summary, our data demonstrated that serum IL-18 levels in patients with HCC correlated with advanced tumor stage classified by Okuda's criteria and the presence of venous invasion. Serum IL-18 level also exhibited an independent predictor of prognosis in patients with HCC. These data suggest that IL-18 contributes an important role in the pathogenesis and disease progression of HCC. If confirmed in additional longitudinal studies, the immuno-modulation of this cytokine may have therapeutic potential in the future.

ACKNOWLEDGMENTS

This study was supported by the Rajadapiseksompoj research grant. We also thank Thailand Research Fund

and Center of Excellence, Viral Hepatitis Research Unit, Chulalongkorn University.

REFERENCES

1 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16
2 **Talwalkar JA**, Gores GJ. Diagnosis and staging of hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S126-S132
3 **Qin LX**, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 193-199
4 **Gracie JA**, Robertson SE, McInnes IB. Interleukin-18. *J Leukoc Biol* 2003; **73**: 213-224
5 **Mühl H**, Pfeilschifter J. Interleukin-18 bioactivity: a novel target for immunopharmacological anti-inflammatory intervention. *Eur J Pharmacol* 2004; **500**: 63-71
6 **Nakanishi K**, Yoshimoto T, Tsutsui H, Okamura H. Interleukin-18 regulates both Th1 and Th2 responses. *Annu Rev Immunol* 2001; **19**: 423-474
7 **Fantuzzi G**, Dinarello CA. Interleukin-18 and interleukin-1 beta: two cytokine substrates for ICE (caspase-1). *J Clin Immunol* 1999; **19**: 1-11
8 **Dinarello CA**. Interleukin-18. *Methods* 1999; **19**: 121-132
9 **Coughlin CM**, Salhany KE, Wysocka M, Aruga E, Kurzawa H, Chang AE, Hunter CA, Fox JC, Trinchieri G, Lee WM. Interleukin-12 and interleukin-18 synergistically induce murine tumor regression which involves inhibition of angiogenesis. *J Clin Invest* 1998; **101**: 1441-1452
10 **Tanaka F**, Hashimoto W, Okamura H, Robbins PD, Lotze MT, Tahara H. Rapid generation of potent and tumor-specific cytotoxic T lymphocytes by interleukin 18 using dendritic cells and natural killer cells. *Cancer Res* 2000; **60**: 4838-4844
11 **Lebel-Binay S**, Berger A, Zinzindohoué F, Cugnenc P, Thiounn N, Fridman WH, Pagès F. Interleukin-18: biological properties and clinical implications. *Eur Cytokine Netw* 2000; **11**: 15-26
12 **Tsuboi K**, Miyazaki T, Nakajima M, Fukai Y, Masuda N, Manda R, Fukuchi M, Kato H, Kuwano H. Serum interleukin-12 and interleukin-18 levels as a tumor marker in patients with esophageal carcinoma. *Cancer Lett* 2004; **205**: 207-214
13 **Pagès F**, Berger A, Henglein B, Piqueras B, Danel C, Zinzindohoue F, Thiounn N, Cugnenc PH, Fridman WH. Modulation of interleukin-18 expression in human colon carcinoma: consequences for tumor immune surveillance. *Int J Cancer* 1999; **84**: 326-330
14 **Takubo T**, Okura H, Kumura T, Nishiki S, Kinoshita Y, Koh K, Ohta K, Yamane T, Hino M, Kamitani T, Tatsumi N. Human IL-18 bioactivity in hematological malignancies with highly elevated serum IL-18 levels. *Acta Haematol* 2000; **103**: 162-164
15 **Günel N**, Coskun U, Sancak B, Hasdemir O, Sare M, Bayram O, Celenkoglu G, Ozkan S. Prognostic value of serum IL-18 and nitric oxide activity in breast cancer patients at operable stage. *Am J Clin Oncol* 2003; **26**: 416-421
16 **Kawabata T**, Ichikura T, Majima T, Seki S, Chochi K, Takayama E, Hiraide H, Mochizuki H. Preoperative serum interleukin-18 level as a postoperative prognostic marker in patients with gastric carcinoma. *Cancer* 2001; **92**: 2050-2055
17 **Tartour E**, Fridman WH. Cytokines and cancer. *Int Rev Immunol* 1998; **16**: 683-704
18 **Wang JM**, Deng X, Gong W, Su S. Chemokines and their role in tumor growth and metastasis. *J Immunol Methods* 1998; **220**: 1-17
19 **Balkwill F**, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 2001; **357**: 539-545
20 **Chau GY**, Wu CW, Lui WY, Chang TJ, Kao HL, Wu LH, King KL, Loong CC, Hsia CY, Chi CW. Serum interleukin-10 but not interleukin-6 is related to clinical outcome in patients with resectable hepatocellular carcinoma. *Ann Surg* 2000; **231**: 552-558
21 **Hattori E**, Okumoto K, Adachi T, Takeda T, Ito J, Sugahara K, Watanabe H, Saito K, Saito T, Togashi H, Kawata S. Possible contribution of circulating interleukin-10 (IL-10) to anti-tumor immunity and prognosis in patients with unresectable hepatocellular carcinoma. *Hepatol Res* 2003; **27**: 309-314
22 **Ren Y**, Poon RT, Tsui HT, Chen WH, Li Z, Lau C, Yu WC, Fan ST. Interleukin-8 serum levels in patients with hepatocellular carcinoma: correlations with clinicopathological features and prognosis. *Clin Cancer Res* 2003; **9**: 5996-6001
23 **Asakawa M**, Kono H, Amemiya H, Matsuda M, Suzuki T, Maki A, Fujii H. Role of interleukin-18 and its receptor in hepatocellular carcinoma associated with hepatitis C virus infection. *Int J Cancer* 2006; **118**: 564-570
24 **Chia CS**, Ban K, Ithnin H, Singh H, Krishnan R, Mokhtar S, Malihan N, Seow HF. Expression of interleukin-18, interferon-gamma and interleukin-10 in hepatocellular carcinoma. *Immunol Lett* 2002; **84**: 163-172
25 **Wang ZY**, Gaggero A, Rubartelli A, Rosso O, Miotti S, Mezzanzanica D, Canevari S, Ferrini S. Expression of interleukin-18 in human ovarian carcinoma and normal ovarian epithelium: evidence for defective processing in tumor cells. *Int J Cancer* 2002; **98**: 873-878
26 **Gately MK**, Renzetti LM, Magram J, Stern AS, Adorini L, Gubler U, Presky DH. The interleukin-12/interleukin-12-receptor system: role in normal and pathologic immune responses. *Annu Rev Immunol* 1998; **16**: 495-521
27 **Kohno K**, Kurimoto M. Interleukin 18, a cytokine which resembles IL-1 structurally and IL-12 functionally but exerts its effect independently of both. *Clin Immunol Immunopathol* 1998; **86**: 11-15
28 **Vagliani M**, Rodolfo M, Cavallo F, Parenza M, Melani C, Parmiani G, Forni G, Colombo MP. Interleukin 12 potentiates the curative effect of a vaccine based on interleukin 2-transduced tumor cells. *Cancer Res* 1996; **56**: 467-470
29 **Murakami S**, Okubo K, Tsuji Y, Sakata H, Hamada S, Hirayama R. Serum interleukin-12 levels in patients with gastric cancer. *Surg Today* 2004; **34**: 1014-1019
30 **Kishimoto T**. The biology of interleukin-6. *Blood* 1989; **74**: 1-10
31 **Wolvekamp MC**, Marquet RL. Interleukin-6: historical background, genetics and biological significance. *Immunol Lett* 1990; **24**: 1-9
32 **Blay JY**, Negrier S, Combaret V, Attali S, Goillot E, Merrouche Y, Mercatello A, Ravault A, Tourani JM, Moskovtchenko JF. Serum level of interleukin 6 as a prognosis factor in metastatic renal cell carcinoma. *Cancer Res* 1992; **52**: 3317-3322
33 **Scambia G**, Testa U, Benedetti Panici P, Foti E, Martucci R, Gadducci A, Perillo A, Facchini V, Peschle C, Mancuso S. Prognostic significance of interleukin 6 serum levels in patients with ovarian cancer. *Br J Cancer* 1995; **71**: 354-356
34 **Nakashima J**, Tachibana M, Horiguchi Y, Oya M, Ohigashi T, Asakura H, Murai M. Serum interleukin 6 as a prognostic factor in patients with prostate cancer. *Clin Cancer Res* 2000; **6**: 2702-2706
35 **Ishikawa M**, Yogita S, Miyake H, Fukuda Y, Harada M, Wada D, Tashiro S. Clarification of risk factors for hepatectomy in patients with hepatocellular carcinoma. *Hepatogastroenterology* 2002; **49**: 1625-1631
36 **Parasole R**, Izzo F, Perrone F, Pignata S, Galati MG, Leonardi E, Castiglione F, Orlando R, Castello G, Esposito G, Gallo C, Daniele B. Prognostic value of serum biological markers in patients with hepatocellular carcinoma. *Clin Cancer Res* 2001; **7**: 3504-3509

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