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Biological significance of serum soluble tumor necrosis factor receptor I in hepatoma patients

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Abstract

AIM: In order to elucidate the biological significance of soluble tumor necrosis factor receptor (sTNF-R) in hepatomas, we observed the differential profiles of serum sTNF-R levels in 83 hepatoma patients and 61 healthy controls.

METHODS: The serum levels of soluble sTNF-R were measured with sandwich enzyme immunoassay.

RESULTS: The mean serum sTNF-R levels were significantly higher in the hepatoma patients than in the controls (2.69 ± 0.79 μg/L *vs* 0.93 ± 0.29 μg/L, $P < 0.001$). The increment correlated well with the stage of the disease, *i.e.* the serum levels of soluble sTNF-RI in the patients with stages III-IV were greater than in those with stages I-II (2.97 ± 0.43 μg/L *vs* 1.74 ± 0.41 μg/L, $P < 0.001$). Additionally, we found that increased sTNF-R level correlated positively with serum alkaline phosphatase ($r = 0.59$), white cell count ($r = 0.43$) and serum globulin ($r = 0.32$), and correlated negatively with serum albumin ($r = -0.71$). Among the hepatoma patients, the frequency of increased serum sTNF-RI level (89.16%) greatly exceeded that of serum AFP (54.22%). Moreover, comparison of 25 patients before and after chemotherapy indicated that patients with a rise in sTNF-R over the therapy course had decreased clinical response (3.39 ± 0.43 μg/L *vs* 2.67 ± 0.34 μg/L, $P < 0.001$).

CONCLUSION: In patients with hepatoma, serum soluble sTNF-R levels correlate well with disease stage and response to chemotherapy. It is reasonable to postulate that this determination can serve as an aid for the detection of these cancers, for follow-up studies, and for assessments of prognosis.

Key words: Liver neoplasms/drug therapy; Receptors, tumor necrosis factor/blood

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INTRODUCTION

Tumor necrosis factors (TNFs) are pleiotrophic cytokines produced primarily by monocytic phagocytes, acting as the primary modifiers of body immune response and initiating multiple effects on cell function[1] by binding to specific, high-affinity cell surface receptors. There are two forms of TNFs, known as TNFα (cachetin) and TNFβ (lymphotoxin). In addition, there are two molecular species of TNF receptors (TNF-Rs)[2]. The cell surface form of TNF-Rs mediates the intracellular signaling of the cellular response to TNF[3]. Each species possesses not only a cell surface form but also two soluble TNF receptor forms, which act to neutralize the biological activities of TNFα- and TNFβ, and are respectively designated as sTNF-RII and sTNF-RI[2]. It has been demonstrated that these two types of TNF-Rs represent the soluble molecules detached from the cell surface receptors.

The physiological effects of sTNF-Rs remain to be elucidated. Both types of receptors are able to bind to TNF *in vitro* and inhibit its biological activity by competing with other cell surface receptors for TNF. It is suggested that shedding of a soluble receptor in response to release of TNF serves as a mechanism for binding, meanwhile inhibiting the immediate binding of the released TNF to surface receptors. This process is also considered as capable of providing protective effects to other cells[4]. It has been reported that low concentrations of TNF binding to soluble receptors can stabilize TNF and augment its activity[5]. In the present study, we measured the circulating level of sTNF-RI in patients with hepatoma and assessed its function and its relationship with the clinical stage of disease and the patients' responses to chemotherapy.

MATERIALS AND METHODS

Subjects

This study included 83 patients with hepatoma (PLC), hospitalized for treatment between October 1994 and July 1995. The mean age was 47.38 ± 16.14 years-old (range, 29-64 years-old). The ratio of males:females was 58:21. All cases were diagnosed by findings from CT scan and/or pathologic biopsy. No patient was

Table 1 Comparison of frequency of increase of sTNF-RI and alpha-fetoprotein

Hepatoma stage	*n*	$\bar{x} \pm s$	Percentage of increased sTNF-RI (*n*)	Percentage of increased AFP (*n*)
Ⅰ-Ⅳ	83	2.62 ± 0.79	89.16% (74)	54.22% (45)
Ⅰ-Ⅱ	24	1.74 ± 0.41	66.67% (16)	45.83% (11)
Ⅲ-Ⅳ	59	2.97 ± 0.43	98.30% (58)	57.63% (34)
Control	61	0.93 ± 0.29		

AFP: Alpha-fetoprotein.

Table 2 Correlations between sTNF-RI and other parameters

	r
Hemoglobin	-0.11
Total white blood cell count	0.43[b]
Albumin	-0.71[b]
Globulin	0.32[a]
Alkaline phosphatase	0.59[b]
Gamma-glutamyltransferase	0.05

[a]$P < 0.05$, [b]$P < 0.01$

Table 3 Mean serum levels of sTNF-RI in 25 hepatoma patients before and after chemotherapy and their relationship to clinical response

Patient no.	Stage	Chemotherapy	Clinical response	sTNF-RI	
				Before	After
1	Ⅳ	A	PR	3.25	3.07
2	Ⅳ	A	PR	3.38	3.11
3	Ⅳ	A	PR	3.15	2.83
4	Ⅳ	V	D	2.95	3.36
5	Ⅲ	A	D	2.33	2.91
6	Ⅲ	A	D	2.97	3.38
7	Ⅳ	A	PR	2.65	2.37
8	Ⅲ	A	D	2.36	2.84
9	Ⅲ	A	D	2.38	2.74
10	Ⅳ	A	D	2.79	3.16
11	Ⅲ	A	PR	2.62	2.53
12	Ⅳ	A	D	3.18	3.46
13	Ⅳ	A	D	3.07	3.23
14	Ⅳ	A	D	3.52	3.93
15	Ⅳ	V	D	3.44	3.51
16	Ⅳ	A	D	3.27	3.67
17	Ⅳ	A	PR	2.96	2.73
18	Ⅲ	A	CR	2.78	2.11
19	Ⅲ	A	PR	2.74	2.53
20	Ⅳ	A	CR	3.25	2.87
21	Ⅳ	V	D	2.79	3.43
22	Ⅳ	V	D	3.54	4.18
23	Ⅲ	V	D	2.62	3.11
24	Ⅳ	V	D	2.88	3.22
25	Ⅳ	V	D	3.85	4.63
	$\bar{x} \pm s$	Patients in remission ($n = 9$)		2.98 ± 0.29	2.67 ± 0.34
		Patients showing deterioration ($n = 16$)		2.99 ± 0.53	3.39 ± 0.43

A: FAPM transcatheter arterial chemoembolization with 5-Fu 1 g, ADM 50 mg, CDDP 100 mg, MMC 10 mg; V: FAM chemotherapy *via* peripheral vein with 5-Fu 500 mg, ADM 40 mg, MMC 8 mg. CR: Complete remission; PR: Partial remission; D: Deterioration.

given steroids or opioids during the investigation. In order to avoid possible interference, the patients with concomitant inflammatory diseases were excluded.

All patients had a detailed medical history and underwent a thorough physical examination. Laboratory tests included complete blood counts, hepatic and renal function tests, and serum alpha-fetoprotein (AFP) measurement. Staging of the hepatomas was done in accordance with the International TNM classification system[6].

The control group consisted of 61 consecutive healthy subjects seen in our outpatient department for a routine check-up. The mean age was 44.24 ± 12.31 years-old (range, 20-70 year-old) and the ratio of males:females was 28:33.

In addition, 25 patients scheduled to undergo chemotherapy were enrolled in the study for evaluation of their data before chemotherapy or 20 d after delivery of the last chemotherapeutic dose.

Methods

Venous blood samples were drawn in the morning, immediately centrifuged and stored at -20 ℃. The serum levels of sTNF-RI were measured using a sandwich enzyme immunoassay kit (Amersham). The measured sTNF-RI concentration was expressed in μg/L units and the sensitivity was 0.01 μg/L. The intra- and inter-assay coefficients of variation were less than 6.85% and 8.73%, respectively.

Serum AFP levels were measured using a double antibody ^{125}I-radioimmunoassay kit (China Institute of Atomic Science), for which 20 μg/L was taken as the normal upper limit in our laboratory.

Statistical analysis

Data were analyzed with the Student's *t*-test. The correlation coefficients were calculated and regression equation analyses were derived appropriately.

RESULTS

The serum concentrations of sTNF-RI in the 83 hepatoma patients and the 61 healthy controls are listed in Table 1.

Among the healthy subjects, the mean concentration ($\bar{x} \pm s$) of sTNF-RI was 0.93 ± 0.29 μg/L. The mean value in the hepatoma patients was 2.97 ± 0.43 μg/L, which was significantly higher than that in the healthy subjects ($P < 0.001$).

The hepatoma patients showed significantly higher serum levels of sTNF-RI. The increment in serum sTNF-RI concentration correlated well with the staging of the cancers, being higher in more advanced cases.

A positive correlation was found between increasing serum sTNF-RI level and alkaline phosphatase level ($r = 0.59$, $P < 0.01$). The increase in sTNF-RI also correlated positively with the white cell counts and the serum globulin concentration, but correlated negatively with the serum albumin concentration. No correlation was found between sTNF-RI and hemoglobin or gamma-glutamyltransferase (Table 2).

Among the cancer patients, only partial correlation was observed between the increase in the serum sTNF-RI and AFP. AFP was elevated (> 20 μg/L) in 45 patients only (40.96%) (Table 2). All the other patients had elevation of the sTNF-RI level; however, this increase was also observed in the other patients with normal serum AFP. Altogether, 74 cancer patients had an increase in sTNF-RI (89.15%). In the chemotherapy group, the sTNF-RI levels measured at the end of the chemotherapeutic schemes were significantly higher (3.39 ± 0.43 μg/L *vs* 2.99±0.53 μg/L, $P < 0.01$) in patients who showed deterioration than in those who showed good responses to the therapies (2.67 ± 0.34 μg/L *vs* 2.98 ± 0.29 μg/L); no difference was seen before the use of chemotherapy (see Table 3).

DISCUSSION

The sTNF-RI level is reportedly increased significantly in a large proportion of patients with hepatoma, and the increment correlates well with the staging of the disease, but the mechanism involved as well as its functional implication remained unknown[7]. We had previously found elevation of the soluble IL-2 receptor in cancer patients[8,9] and speculated that the observed excess of TNF receptors in the serum of cancer patients were, at least in part, produced by the tumor cells. Some authors[10] reported that various tumor-derived cell lines could produce the soluble TNF receptors spontaneously in culture. This finding supports our speculation. But, there is still much diversity among the ideas related to the presumption that the receptors could be produced by a normal cell population in response to the tumor process, which represents an alternative possibility. Furthermore, this suggests that the two possibilities are not mutually exclusive. The excess of soluble receptors reflects an over-production, both by the malignant cells and by the normal cells, and this process may be related to the immune system. There is evidence from *in vitro* studies that indicates the tumor cells have a greater tendency than the normal cells to shed the soluble form of their cell surface proteins, apparently as a result of enhancing the cleavages of the cell surface

molecules[7]. This process is similar to that seen in cases of sIL-2R[8].

It has been shown that sTNF-R can compete with the cell surface receptor for TNF, thereby interfering with its cytokine function[7]. TNF can have a destructive effect on tumor cells, as shown in some experimental cancer models[11]; as such, the elevated levels of sTNF-R in cancer patients may represent a tumor "escape" mechanism from the suppressive effect of sTNF-R. It is possible that the increased binding of receptors to other cytokines in the sera of cancer patients may contribute to the general suppression of certain immunologic functions that has been observed in advanced cancer patients.

An increase in the level of serum sTNF-RI is certainly not a distinctive feature of cancer, since it also occurs in a variety of other inflammatory and autoimmune diseases[12]. Nevertheless, sTNF-RI might have a diagnostic value, since after exclusion of an inflammatory or autoimmune disease elevated levels of soluble TNF receptor may be suggestive of the presence of a malignant process. In our study, increased serum sTNF-RI was found in a cancer condition involving the gastrointestinal tract, other types of these cancers include gastric, pancreatic and colorectal. Such increased serum sTNF-RI has also been reported in cases of breast cancer[7]. The frequency of increased serum sTNF-RI in our current hepatoma patient population (90%) is similar to that of sIL-2R (85%) and also higher than that of serum AFP (54%), which is a common tumor marker of the hepatoma.

The present investigation seems to suggest that the clinical response to chemotherapy may be associated with normalization of the serum sTNF-RI level, whereas further increase would indicate deterioration. However, the patient series examined is as yet not large enough to draw a proper conclusion. Further investigation will be required to delineate the biological and prognostic significance for hepatoma patients. So far, there is no evidence that the type of therapy and the type of histologic pattern may have any impact on sTNF-RI release.

Our results suggest, however, that the serum sTNF-RI concentration in patients with hepatoma correlates well with the staging of the disease and also may serve as an aid for the detection of the cancers, for follow-up studies, and for assessments of prognoses.

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