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Increased intratumoral IL-17-producing cells correlate with poor survival in hepatocellular carcinoma patients☆

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***Background/Aims*: To characterize IL-17-producing cells, a newly defined T helper cell subset with potent pro-inflammatory properties, in hepatocellular carcinoma (HCC) and to determine their prognostic values.**

***Methods*: One hundred and seventy-eight HCC patients were enrolled randomly. Distribution and phenotypic features of IL-17-producing cells were determined by flow cytometry and/or immunohistochemistry.**

***Results*: Compared with corresponding non-tumor regions, the levels of Th17 cells were significantly increased in tumors of HCC patients ($P < 0.001$). Most intratumoral Th17 cells exhibited an effector memory phenotype with increased expression of CCR4 and CCR6. Intratumoral IL-17-producing cell density was associated with overall survival (OS, $P = 0.001$) and disease-free survival (DFS, $P = 0.001$) in HCC patients. Multivariate Cox analysis revealed that intratumoral IL-17-producing cell density was an independent prognostic factor for OS (HR = 2.351, $P = 0.009$) and DFS (HR = 2.256, $P = 0.002$). Moreover, the levels of intratumoral Th17 cells were positively correlated with microvessel density in tumors ($r = 0.616$, $P = 0.001$).**

***Conclusion*: Accumulation of intratumoral IL-17-producing cells may promote tumor progression through fostering angiogenesis, and intratumoral IL-17-producing cell could serve as a potential prognostic marker and a novel therapeutic target for HCC.**

***Keywords*: Th17 cells; Hepatocellular carcinoma; Prognosis; Angiogenesis; Inflammation**

1. Introduction

Hepatocellular carcinoma (HCC) is the fifth most common cancer, and its incidence is increasing worldwide due to the dissemination of Hepatitis B (HBV) and Hepatitis C (HCV) virus infection [1]. HCC is characterized by progressive development, high postsurgical recurrence and extremely poor prognosis. The dismal outcome has been attributed to the highly vascular nature of HCC, which increases the propensity to spread and invade into neighboring or distant sites [1–3].

Tumor progression has been recognized as the product of an evolving crosstalk between different cell types within the tumor [4]. HCC is usually present in inflamed fibrotic

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Abbreviations: AF, Alexa Fluor; DFS, disease-free survival; FITC, fluorescein isothiocyanate; HCC, hepatocellular carcinoma; MVD, microvessel density; NIL, non-tumor-infiltrating lymphocytes; OS, overall survival; PBMC, peripheral blood mononuclear cells; PE, phycoerythrin; PerCP, peridin chlorophyll; TIL, tumor-infiltrating lymphocytes.

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and/or cirrhotic liver with extensive lymphocyte infiltration due to chronic viral infection. Thus, the immune status at tumor site can largely influence the biologic behavior of HCC [1,5]. Recent studies have shown that intratumoral regulatory T cells (Tregs) are associated with HCC invasiveness, and the balance of regulatory and cytotoxic T cells is a promising independent predictor for recurrence and survival in HCC [6]. These findings are in accordance with the general view that tumor microenvironment induces tolerance [7,8]. However, many cancers arise at the site of chronic inflammation and inflammatory mediators are often produced in tumors [9,10]. In HCC patients, increased HLA-DR$^+$ cells in liver are associated with metastatic phenotype [5]. We have recently found that most macrophages in peritumoral region express high level of HLA-DR [11,12] and that the density of these activated cells is associated with vascular invasion and poor prognosis in HCC. These results strongly indicate that, besides inducing immune tolerance, tumors may also reroute the pro-inflammatory immune response into a tumor-promoting direction, although the relative mechanism remains largely unknown.

CD4$^+$ T helper (Th) cells play a central role in orchestrating host immune responses through their capacity to help other cells of the immune systems. Classically, Th1 cells produce IFN-γ to enhance antimicrobial and antitumor cytotoxic responses, whereas Tregs suppress T-cell immunity in both physiologic and disease statuses [7,13,14]. More recently, a novel IL-17-producing CD4$^+$ T helper cell subset termed Th17 cells has been described. Th17 cells have potent pro-inflammatory properties and play an active role in inflammation and autoimmune diseases [15–19]. Much of the inflammatory damage previously ascribed to Th1 response is now understood to depend on IL-17 and IL-23, the cytokine important for supporting Th17 response *in vivo* [18,19]. Strikingly, IL-23 may promote tumor incidence and growth and IL-17 has been shown to promote tumor growth by increases in angiogenesis and intratumoral infiltration of phagocytes in mouse [20–23]. Moreover, an increase in IL-17-producing cells was also found in both peripheral blood and tumor tissues from cancer patients with advanced stages [24]. Although these data suggest a potential impact of Th17 cells on tumor, the nature and role of Th17 cells in human tumor progression remain unknown.

The present study showed that Th17 cells were enriched in tumors of HCC patients. Intratumoral Th17 cells exhibited a CD45RO$^+$CD62L$^-$CCR7$^-$ effector memory phenotype and highly expressed CCR4 and CCR6. Moreover, increased intratumoral IL-17-producing cell density predicted poor prognosis in HCC patients and the levels of intratumoral Th17 cells were correlated with microvessel density in tumor.

2. Patients and methods

2.1. Patients and specimens

Tumor or peripheral blood samples were obtained from 178 patients with pathologically confirmed HCC at the Cancer Center of Sun Yat-Sen University. None of the patients received anticancer therapy before sampling. Individuals with concurrence of autoimmune disease, HIV and syphilis were excluded. Blood samples from 51 patients as well as fresh tumor and non-tumor (at least 3-cm distant from the tumor site) tissues from 28 patients (Group 1) who received therapy between 2007 and 2008 were used for isolation of peripheral blood mononuclear cells (PBMC), tumor-infiltrating lymphocytes (TIL) and non-tumor-infiltrating lymphocytes (NIL) (including paired blood and tissue samples from 9 patients). Hundred and eight patients (Group 2) who received curative resection, defined as complete macroscopic removal of the tumor, between 1999 and 2003 with follow-up data were enrolled in survival analysis. Surgical procedures were performed as previously described [25,26]. Patients with confirmed recurrence received further treatment, such as surgery, radiofrequency ablation, percutaneous ethanol injection, percutaneous microwave coagulation therapy or transcatheter arterial chemoembolization [26,27]. Overall survival (OS) was defined as the interval between the dates of surgery and death. Disease-free survival (DFS) was defined as the interval between the dates of surgery and recurrence or the last follow-up if no recurrence was observed. Clinical stages of tumors were determined according to the TNM classification system of International Union Against Cancer (Edition 6). Tumor differentiation is graded according to Edmondson–Steiner classification. Clinical char-

Table 1
Clinical characteristics of 178 patients with HCC.

Variable	Group 1	Group 2
Cases (*n*)	70	108
Age, years (median, range)	49, 16–78	46, 17–71
Gender (male/female)	64/6	95/13
HBsAg (negative/positive)	2/68	9/99
Cirrhosis (absent/present)	24/46	22/86
FP, ng/ml (⩽25/>25)	28/42	33/75
Tumor multiplicity (solitary/multiple)	44/26	92/16
Tumor size, cm (⩽5/>5)	36/34	39/69
Tumor differentiation (I–II/III–IV)	25/25[A]	61/47
Child-Pugh classification (A/B)	65/5	104/4
Vascular invasion (absent/present)	61/9	101/7
TNM stage (I–II/III–IV)	47/23	82/26

HBsAg, hepatitis B surface antigen; AFP, α-fetoprotein.
[A] Information about tumor differentiation is not available for 20 patients who did not undergo liver resection.