Effects of Radiofrequency Ablation on Lymphocyte Subsets and Type 1/Type 2 T Cell Subpopulations in Patients with Hepatocellular Carcinoma

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ABSTRACT

Objective: To evaluate whether radiofrequency ablation (RFA) might have an influence on immune status in hepatocellular carcinoma (HCC) patients.

Methods: We measured the T lymphocytes, B lymphocyte and NK cells, and determined the population of Th1, Th2, Tc1 and Tc2 of peripheral blood samples taken from 26 HCC patients before and after RFA.

Results: The proportion of Type1 cells (Th1 and Tc1) and NK cells were significantly increased after RFA, especially in patients of the following subgroups: male, age>55 years, pathological grade I-II tumor, clinical stage I-II or Child-Pugh A and B.

Conclusion: Type1 cells and NK cells in HCC patients were increased in a short period after RFA.

Key words: Hepatocellular carcinoma; Radiofrequency ablation; Lymphocyte subsets; T-lymphocyte subsets; Th1/Th2; Tc1/Tc2

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common and severe malignancies in China. Since radiation and chemotherapy are not effective for HCC, surgical resection provides the best potentially curative option. Unfortunately, over 70% patients of HCC are not resectable due to the size, site, number of tumor, and poor liver function etc.. During the past decade, the local mesenchymal treatments have been developed rapidly, of which radiofrequency ablation (RFA) has shown big potential advantage for HCC patients with above characteristics[1-2].

In patients with advanced carcinoma, depressed systemic immune responses, including decreased cellular immunity, have been observed. Furthermore, studies have shown that some therapies, such as surgical procedure and radiation therapy were insufficient for the patients to overcome or even to aggravate the immunological depression[3]. Helper T type 1 (Th1) cells, Th2,cytotoxic T (Tc1) cells and Tc2 cells, and natural killer (NK) cells play important roles in the complex immunoregulation. As well known, Th1 and Tc1 cells (Type1 cells) promote cellular immunity through producing Type1 cytokines, such as interferon (IFN)-γ or interleukin (IL)-2. Th2 and Tc2 cells (Type2 cells) suppress cellular immunity through increased production of Type2 cytokines, such as interleukin (IL)-4 and IL-10. NK cells play an important role in host anticancer defense mechanisms, inducing the nonspecific antitumor immune response. Recent studies have demonstrated that an elevated level of Type2 cytokines contributes to the ability of cancer cells to escape immunosurveillance[4]. However, the influence of RFA treatment on the systemic cellular immunological parameters in HCC patients has not been reported. In present study, we conducted an immunological assessment utilizing flow cytometry as described previously[5], a procedure for analyzing cell surface and intracellular antigens simultaneously at

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the single cell level, in HCC patients underwent RFA treatment.

MATERIALS AND METHODS

Patients

From October 2003 through December 2004, a total of 57 consecutive HCC patients were diagnosed in our hospital by ultrasound-guided fine-needle aspiration biopsy or by computed tomography(CT), positive serum alpha-fetoprotein (AFP) and regular follow-up for at least 3 months. The patients were recruited into the study according to the following criteria: coinciding with the indication of undergoing curative RFA treatment; not receiving surgical resection and other coagulation regional therapy or systemic chemotherapy before or within 1 month after RFA; without any disease of immune dysfunction; not taking medications known to affect the immune system before or within 1 month after RFA. Among the 57 patients, 26 patients were eligible into this study. The median age was 58.3 years (range from 31 to 71 years). Eighteen male and 8 female patients were included. Among the 26 HCC patients, 19 were clinically classified at stage I or II, 3 at stage III and 4 at stage IV according to UICC-TNM system. Twenty of the 26 patients had liver function of Child-Pugh Class A; 4 of Class B; and 2 of Class C. Tumors in 21 of the 26 patients were pathologically graded at I or II, 5 at III or IV. The average diameter of HCC was 3.1 ± 1.0cm (range, 1.5-5.0cm).

RFA Treatment Procedure and Follow Up

All RFA treatments were performed with conscious sedation or general anesthesia. Conscious sedation was induced with intravenous administration of 2.5~5.0mg of midazolam (Roche; Basel, Switzerland) and 50-100 μg of fentanyl (Fentaini; Renfu, Yichang, China). Local infiltration anesthesia was induced by 5-15 ml of 1% lidocaine (Liduokayin; Yimin, Beijing, China). The RFA system used in this study was a 460-KHz generator unit (Model RITA 1500). The electrode contained nine hook-shaped prongs (Starburst XL; RITA Medical Systems) and enabled the ablation of a 5.0cm region. Tumors larger than 3.5cm were treated with multiple overlapping ablations according to the protocol of Dr. MH Chen[6]. The safe margin in ablation procedure enveloped the entire tumor as well as a 0.5-1.0cm margin of surrounding normal hepatic tissue.

To detect evidence of locoregional and/or systemic tumor recurrence, patients received contrast-enhanced CT, serial assessment of liver function and serum tumor marker (AFP) examination 1 to 2 months after RFA. Then patients were seen regularly in the outpatient clinic (every 3 months). Additional diagnostic procedures were performed if indicated. Follow-up was completed for all patients. The median follow-up duration from treatment was 8 months (range 3-14 months)

Blood Samples

Fasting heparinized blood specimens (5ml) were collected in the morning by elbow venipuncture before RFA and 1 month after RFA treatment. The samples were kept at room temperature and the process of analysis was performed within 4 h.

Reagents and Monoclonal Antibodies

Phorbol 12-myristate 13-acetate(PMA), ionomycin and HYQ RPMI-1640 (1×) (HYClone, without L-Glutamine) were purchased from Sigma (St. Louis, MO, USA). Golgistop, Permeabilizing Solution (Perm) and FACS Lysing Solution were obtained from Becton Dickinson (BD Biosciences, San Jose, USA).

The following reagents were purchased from Becton Dickinson (BD Biosciences, San Jose, USA), including Fluorescein isothiocyanate (FITC)-conjugated anti-IFN-γ/phycoerythrin (PE)-conjugated anti-IL-4-specific mAbs, PE-conjugated mouse IgG1/FITC-conjugated mouse IgG2α (used as controls); Peridinium chlorophyll protein (PerCP)-conjugated anti-CD3-specific mAbs, allophycocyanin (APC)-conjugated anti-CD8-specific mAb; FITC-conjugated anti-CD4/PE-conjugated anti-CD8/ PerCP-conjugated anti-CD3-specific mAbs, FITC-conjugated anti-CD3/PE-conjugated anti-CD19-specific mAbs, FITC-conjugated anti-CD3/PE-conjugated anti-CD16+56-specific mAbs, and FITC-conjugated mouse IgG1/PE-conjugated mouse IgG1/ PerCP-conjugated anti-CD3-specific mAbs (used as controls).

Lymphocyte Subsets

Heparinized peripheral whole blood (100μl) was collected into Facon tubes for analyzing T lymphocytes, B, or NK cells. The T lymphocytes including CD3, CD8 and CD4 cells were analyzed by incubating the cells with 10μl anti-$CD4^{FITC}$/$CD8^{PE}$/ $CD3^{Percp}$ three-color fluorescent mAb for 20 min at RT in the dark. For analyzing B cells, 10μl anti-$CD3^{FITC}$/ $CD19^{PE}$ two-color was used, and for NK cells, 10μl anti-$CD3^{FITC}$/$CD16+56^{PE}$ fluorescent mAb was used. A negative control was included by incubating the