Clinical Significance of Serum Soluble Interleukin 2 Receptor-α in Esophageal Squamous Cell Carcinoma

Liang-Shun Wang,2 Kuan-Chih Chow, Wing-Yin Li, Chia-Chuan Liu, Yu-Chung Wu, and Min-Hsiung Huang

Division of Chest Surgery, Departments of Surgery [L-S. W., C. C. L., Y-C. W., M-H. H.] and Pathology [W-Y. L.], Veterans General Hospital in Taipei and National Yang-Ming University, Taipei, and Department of Medical Research, China Medical College Hospital, Taichung, 11217 Taiwan, Republic of China. Phone: 886-2-2875-7060; Fax: 886-2-873-1488; E-mail: lswang@vghtpe.gov.tw.

ABSTRACT

Although the serum level of soluble interleukin-2 receptor α (sIL-2Rα) has been shown to correlate with progression and prognosis of several cancers, data to support its clinical significance to esophageal squamous cell carcinoma (ESCC) are limited. This study was conducted to assess the prognostic value and source of sIL-2Rα in patients with ESCC. From January 1986 to June 1997, 125 patients with histopathologically confirmed ESCC were enrolled for study. Ninety-three patients underwent en bloc esophagectomy, and 32 patients with unresectable tumor underwent palliative surgery. Four (4.3%; 4 of 93) patients died of surgical complications. Serum levels of sIL-2Rα were measured by ELISA. Expression of IL-2Rα, IL-2Rβ, and IL-2Rγ in the pathological section was determined, respectively, by immunohistochemistry (IHC) and in situ hybridization (ISH). Compared with the healthy control group (1020 ± 476 pg/ml, n = 103), ESCC patients tended to have significantly higher serum sIL-2Rα concentrations (1424 ± 798 pg/ml, n = 121). The sIL-2Rα level was correlated with age, Tumor-Node-Metastasis classification, tumor stage, reading score of the IHC staining, and survival but not with the pathological grade or lymphovascular invasion. Prognosis was worse for patients with high sIL-2Rα levels (≥1500 pg/ml) than for those with low serum sIL-2Rα levels (<1500 pg/ml; P = 0.0209). It can be used as an independent prognostic factor of ESCC. In the pathological sections, expression of IL-2Rα, IL-2Rβ, and IL-2Rγ was detected in 17 (18.1%), 83 (92.2%), and 83 (89.2%) cases, respectively, by IHC, and the message of IL-2Rα was identified in tumor cells by ISH in 30.1% (28 of 93) of the cases. Serum concentrations of sIL-2Rα are frequently elevated in ESCC patients and are correlated with disease progression and survival. These data indicate that, in addition to activated T cells, cancer cells could be an important source of sIL-2Rα in ESCC patients.

INTRODUCTION

ESCC3 is common in Mainland China and Taiwan (1–3). Although surgery provides a chance of cure for early-stage esophageal malignancies, most of the patients present with advanced disease. The poor prognosis is further compounded with frequent relapse and early metastasis (4). Therefore, it is important to detect disease progression and metastasis as early as possible to improve timely treatment and improve survival.

Several recent studies have shown that TNM stage and the number of diseased lymph nodes are two important factors associated with the prognosis of ESCC (5–9). Although these two factors can only be assessed during surgery, they are not applicable for monitoring disease advancement and the potential of metastasis. On the other hand, serum biomarkers are often associated with the biological behavior of cancer cells. The prognostic measure of a serum biomarker that can reflect the concerted interaction between the tumor and the host immune system may provide scientific insight to improve the therapeutic strategy. However, the serum biomarkers that can be used as complementary prognostic factors are very few for patients with ESCC.

IL-2, a glycoprotein produced by the activated T cells, can promote the growth of T cells, B cells and natural killer cells by binding to the IL-2R. On the activated T cells, IL-2R consists of three components: α chain (Ka = 10⁻⁸ M), β chain (Ka = 10⁻⁷ M), and γ chain (Ka undetectable; Ref. 10, 11). Only α chain and β chains can bind to IL-2; the γ chain alone does not bind to IL-2. Nevertheless, both β and γ chains are required for transcription of proliferative signals. On the resting T cell, the heterodimeric IL-2Rβγ chain (Ka = 10⁻⁸ M) is constitutively expressed. Once activated by IL-2, T cells start to synthesize IL-2Rα. The newly synthesized IL-2Rα chain joins IL-2Rβ and IL-2Rβγ to form the membrane-bound heterodimeric IL-2Rαβ (Ka = 10⁻¹⁰ M) or heterotrimeric receptor IL-2Rαβγ (Ka = 10⁻¹¹ M). Expression of IL-2Rα is therefore a landmark of T-cell activation. However, a truncated form of IL-2Rα, sIL-2Rα, that has only an extramembranous domain can also be simultaneously detected in the serum (11–14). The affinity of

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2 To whom requests for reprints should be addressed, at Division of Thoracic Surgery, Department of Surgery, Veterans General Hospital in Taipei, #201 Section 2, Shih-Pai Road, Taipei 11217, Taiwan, Republic of China. Phone: 886-2-2875-7060; Fax: 886-2-873-1488; E-mail: lswang@vghtpe.gov.tw.

3 The abbreviations used are: ESCC, esophageal squamous cell carcinoma; TNM, Tumor-Node-Metastasis; IL, interleukin; IL-2R, IL-2 receptor; sIL-2Rα, soluble IL-2Rα; IHC, immunohistochemistry; ISH, in situ hybridization.
sIL-2Rα to bind to IL-2 is similar to that of the membrane-bound α chain (15, 16).

Clinically, serum sIL-2R levels tend to increase in patients with tuberculosis and autoimmune diseases (17). In addition, an elevated serum concentration of sIL-2Rα is also suggested to correlate with adverse prognosis in patients with nasopharyngeal carcinoma (18, 19), colorectal cancer (20, 21), breast cancer (22), ovarian cancer (23–25), gastric cancer (26, 27), lung cancer (28, 29), and leukemia (30, 31). It is worth noting that in these malignancies the increased sIL-2Rα levels not only are associated with disease activities and treatment response but also indicate the pathogenetic significance in disease progression.

In this study, we measured the serum sIL-2Rα levels in patients with ESCC to evaluate its relationship with tumor stage, lymph node metastasis, depth of tumor invasion, pathological findings (grades and lymphovascular invasion), and survival. We also identified the prospective source of IL-2R in the pathological sections by IHC and ISH.

**PATIENTS AND METHODS**

**Human Sera and Tissue Specimens.** From January 1986 to June 1997, 125 consecutive patients with histopathologically proven ESCC were enrolled for study. The average age was 64.5 ± 10.7 years, and the ratio of male:female was 40:3. Tumor stage was classified according to the TNM system (32). Medical Ethical Committee approved the protocol, and the written informed consent was obtained from every patient before surgery. Extensive preoperative measures including esophagoscopy with biopsy, esophagogram, chest radiography, sonograms of abdomen and neck, computed tomography of the chest, and radionuclide bone scanning were followed to determine the need of surgery. Patients with resectable tumor (n = 93) underwent en bloc esophagectomy with locoregional lymphadenectomy through right thoracotomy, laparotomy with reconstruction using the stomach through a retrosternal route, and cervical esophagogastrectomy (33). For patients at stage Ib or beyond, concurrent chemoradiotherapy was applied after surgery (4). Patients with unresectable tumor (n = 32) received chemoradiotherapy after the installation of feeding jejunostomy or bypass procedure. None of these patients received neoadjuvant therapy. After treatment, all patients were followed regularly. Four patients (4.3%) died of cardiopulmonary complication after surgery and were excluded from the prognosis analysis. Serum samples were obtained from each patient at the time of diagnosis. Serum samples from 103 healthy individuals with equivalent distribution of age and sex were used as normal controls. After centrifugation of the peripheral blood, serum samples were stored at −20°C until assayed.

**ELISA.** The serum level of sIL-2Rα was measured with Quantikine human IL-2 sRα (R&D Systems, Inc., Minneapolis, MN). Briefly, diluted serum (1:4) and peroxidase-conjugated polyclonal antibody specific to IL-2Rα was added to each well having been precoated with monoclonal antibody specific to IL-2Rα. After incubation at room temperature for 3 h, the reaction solution was aspirated, and the microtiter plate was
The concentration of sIL-2R was determined by: $\text{P} = 0.0209$.

<table>
<thead>
<tr>
<th>Table 2 Levels of serum sIL-2Rα in patients with ESCC</th>
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<tbody>
<tr>
<td>Category</td>
</tr>
<tr>
<td>Normal control ($n = 103$)</td>
</tr>
<tr>
<td>ESCC ($n = 121$)</td>
</tr>
<tr>
<td>Tumor stage</td>
</tr>
<tr>
<td>Stage I ($n = 11$)</td>
</tr>
<tr>
<td>Stage II ($n = 30$)</td>
</tr>
<tr>
<td>Stage III ($n = 44$)</td>
</tr>
<tr>
<td>Stage IV ($n = 36$)</td>
</tr>
<tr>
<td>Lymph node involvement</td>
</tr>
<tr>
<td>Negative ($n = 38$)</td>
</tr>
<tr>
<td>Positive ($n = 83$)</td>
</tr>
<tr>
<td>Distant metastasis</td>
</tr>
<tr>
<td>Negative ($n = 85$)</td>
</tr>
<tr>
<td>Positive ($n = 36$)</td>
</tr>
<tr>
<td>Tumor status</td>
</tr>
<tr>
<td>$T_1$ ($n = 13$)</td>
</tr>
<tr>
<td>$T_2$ ($n = 11$)</td>
</tr>
<tr>
<td>$T_3$ ($n = 66$)</td>
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<tr>
<td>$T_4$ ($n = 31$)</td>
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<tr>
<td>Cell differentiation</td>
</tr>
<tr>
<td>Well ($n = 12$)</td>
</tr>
<tr>
<td>Moderate ($n = 58$)</td>
</tr>
<tr>
<td>Poor ($n = 11$)</td>
</tr>
<tr>
<td>Lymphovascular invasion</td>
</tr>
<tr>
<td>Negative ($n = 60$)</td>
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<tr>
<td>Positive ($n = 19$)</td>
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</tbody>
</table>

$^a$ - $P$ was determined by: $^a$ Student’s t-test (two-tailed); or $^c$ one-way analysis of variance test.

washed with wash buffer four times. Positive reaction was identified by developing with chromogen solution containing 3,3’,5,5’-tetramethylbenzidine at room temperature for 20 min. The concentration of sIL-2Rα was then determined with concomitant calibration of standard IL-2Rα by reading at A450 nm (MRC; Dynatech Laboratories, Inc., Chantilly, VA). The individual sample was done in duplicate. Samples with overscaled readings were further diluted before measurement.

Immunohistochemistry and Slide Evaluation. Antibodies used for IHC were specific to IL-2Rα (Dako, Kyoto, Japan), IL-2Rβ, and IL-2Rγ (Santa Cruz Biotechnology, Santa Cruz, CA). Immunological staining was performed by an immunoperoxidase method (34). Aminoethyl carbazole was used as chromogenic substrate and crystal precipitate was identified as positive staining. Sample sections were counterstained with hematoxylin, and slides were mounted with glycergelatin. Each batch had a positive and a negative control to ensure quality. Slides were evaluated as described previously (35). Briefly, each slide was evaluated randomly at four areas that contained tumor cells and was photographed. In each case, normal epithelial area of the esophageal tissue served as internal negative control. Slides were read by two independent pathologists having no knowledge of their clinicopathological status. The signal of stain was scored on a 0 to 2 plus subjective scale with 0 = no expression, 1+ = intermediate expression, and 2+ = strong expression. Four areas were examined on three slides from each patient, resulting in 12 scores. An average was taken as the final reading. After judging specificity from the internal negative control, an average score $>0.3$ was defined as positive for the expression of IL-2Rα and otherwise as negative.

ISH. A nonisotopic method with FITC-labeled IL-2Rα antisense oligonucleotides was used to detect the expression of IL-2Rα mRNA (24). The probe sequences are as follows: 5’-GGAAACCTCTCTTGCATCAGTCAACATGG-3’ (IL-2Rα mRNA, nucleotides 337–306); 5’-AGTGGCGAGCTTGTCGATTGACCTTGGTTGT-3’ (KCa2, nucleotides 439–408); 5’-AGACACCTCTCAAGAGCCTCTGTCA-GACCC-3’ (KCa3, nucleotides 681–651); 5’-CACTCGAAGGGAGCGGTTGACAGGAG-3’ (IL-2Rα mRNA, nucleotides 946–918). Hybridization products were visualized by using alkaline phosphatase-conjugated polyclonal antibodies to FITC (Amersham International, Buckinghamshire, United Kingdom) and chromogen nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl phosphate (Sigma Chemical Co., St. Louis, MO). Positive staining was identified microscopically as brownish blue granules at the site of hybridization.

Statistical Analysis. The results are expressed as mean ± SD. The relationship between serum sIL-2Rα level and each of the clinicopathological parameters (age, lymph node involvement, distant metastasis, cell differentiation, lymphovascular invasion, and tumor stage) was analyzed by chi-square test. When any analysis cell had fewer than five cases, the Fisher’s exact test was used. The statistical difference between groups A and B in each clinicopathological category was determined by Student’s t test (two-tailed) or ANOVA. Correlation between serum sIL-2Rα level and the immunohistochemical reading score of IL-2Rα expression in ESCC patients was analyzed by a simple curve fit test. Survival curves were plotted with method of Kaplan-Meier. The statistical difference in survival between different groups was compared by the log-rank test. Survival correlation with the prognostic factors was further investigated by multivariate analysis using Cox proportional hazards model with backward stepwise likelihood ratio. Statistical analysis was performed using SPSS statistical software (Chicago, IL). Statistical significance was assumed for $P < 0.05$. Fig. 1 Survival for patients with ESCC in relation to their serum sIL-2Rα levels. The cutoff value of serum sIL-2Rα concentration for dividing group A and B was defined at 1500 pg/ml that represents the mean plus 1 SD measured from the healthy control subjects. The survival analysis between group A (sIL-2Rα = 1500 pg/ml) and group B (sIL-2Rα, <1500 pg/ml) was assessed by the Kaplan-Meier method, and the survival difference between groups was compared by the log-rank test ($P = 0.0209$).
RESULTS

Serum sIL-2Rα Level and Clinicopathological Features in ESCC Patients. Serum sIL-2Rα was 1020 ± 476 pg/ml in normal healthy controls (range, 287.8–1621 pg/ml; n = 103) and was significantly higher in patients with ESCC (1424 ± 798 pg/ml; n = 121; P < 0.005). It was above the normal range in 70.2% (85 of 121) of ESCC patients before surgery, and 31.4% (38 of 121) of them had >1500 pg/ml, the mean plus 1 SD as determined from the control. Using 1500 pg/ml as the cutoff value, these ESCC patients were then divided into group A (n = 38) as those with the higher level (>1500 pg/ml; mean, 2357.6 pg/ml; range, 1507.6–4601 pg/ml) and group B (n = 83) as those with lower level (<1500 pg/ml; mean, 996.2 pg/ml; range, 253.6–1474 pg/ml). χ² analysis showed that the preoperative serum sIL-2Rα levels correlated well with tumor stage, the depth of tumor invasion (T status), lymph node involvement (N status), distant metastasis (M status), and age (Table 1). Higher sIL-2Rα levels were related to disease progression (Table 2). Serum levels of sIL-2Rα were significantly higher in patients with lymph node metastasis or advanced-staged tumors than in those without lymph node involvement or early-stage tumors. However, although the patients were grouped by their histopathological findings (pathological grade or lymphovascular invasion), no more differences could be found in their serum sIL-2Rα levels (Tables 1 and 2).

Correlation of Serum sIL-2Rα Level with the Prognosis of ESCC Patients. The overall cumulative survival rates of our patients were 41% at 2 years and 19% at 5 years. In view of the serum sIL-2Rα level, group A patients seemed to have much worse prognosis than group B patients (P = 0.0209; Fig. 1). The cumulative 2-year survival rate for group A patients was 29.8% and for group B patients was 47.6%. The median survival for group A was 8.8 months, and for group B was 21.3 months. Among patients who had persistently high sIL-2Rα levels or a marked increase of sIL-2Rα level within a short interval (1–6 months) after esophagectomy, distant metastasis of cancer (8 of 13) was found frequently. On the other hand, patients with a low preoperative level of sIL-2Rα (n = 12) or having sIL-2Rα levels decreasing after esophagectomy (n = 5) could remain disease free for 12–23 months (17 of 17). As aforementioned, the depth of tumor invasion, lymph node metastasis, distant nodal or organ metastasis, and age correlated well with the sIL-2Rα level. The further multivariate analysis also showed them as the independent factor of patient survival (lymph node metastasis, distant nodal or organ metastasis, and age).
Expression of IL-2Rs in the Pathological Sections. IHC and ISH were used to determine IL-2R expression in the pathological sections. By IHC, expression of IL-2Rα (Fig. 2A), IL-2Rβ (Fig. 2B), and IL-2Rγ (Fig. 2C) was noticed, respectively, in 28 (30.1%), 83 (89.2%), and 83 (89.2%) cases of pathological sections (n = 93). However, the hybridized product of IL-2Rα mRNA was detected in tumor cells only in 18.3% (17 of 93) of the cases (Fig. 2D). Interestingly, all IL-2Rα mRNA positive cases were identified to have a high level of serum sIL-2Rα (>1500 pg/ml). Cricket Graph software was used to estimate the relationship between serum sIL-2Rα level and the reading score of immunohistochemical IL-2Rα expression (Fig. 3) and found significant correlation between both parameters, shown by a simple curve fit (R² = 0.732).

DISCUSSION

Several studies have shown that the clinical response of esophageal carcinoma is correlated with tumor stage and lymph node metastasis (5, 9). We have also found elevated serum IL-6 not uncommonly in ESCC patients, and its association with tumor stage and lymph node metastasis in turn renders a poor prognostic measure (36). The present study further supports elevated serum sIL-2Rα in ESCC patients and its correlation with poor prognosis.

In recent years, patient immune competence has emerged as a clinically important parameter closely associated with the treatment outcome of the cancer patient (37–39). Early tumor recurrence and disease progression are believed to have something to do with the rapid growth of tumor cells and the evident lymphovascular invasion that may escape the immune surveil-

lance. Although suppression of the immunoreactivity has been observed (40), there is little description on the interaction between immune defense and disease status of ESCC patients.

A study by Jablonska et al. (41) has demonstrated a significantly higher serum sIL-2Rα level in ESCC patients than in control groups. Because sIL-2Rα can potentially bind to IL-2, high sIL-2Rα levels in cancer patients may therefore contribute as a poor prognostic factor to decrease IL-2-mediated immunoreactivities of lymphocytes. It has been suggested that the sIL-2Rα level be useful for prognosis estimation and for monitoring disease progression. According to our findings, the serum sIL-2Rα level not only increased substantially in ESCC patients but also correlated well with TNM factors and tumor stage. High serum levels of sIL-2Rα at diagnosis may imply higher a relapse rate and shorter survival. However, our results did not demonstrate any correlation of serum sIL-2Rα level with histopathological grade or lymphovascular invasion.

As noted previously, binding of IL-2 can promote proliferation of immune cells and biosynthesis of IL-2. Immune cells can then secrete sIL-2Rα to elevate the serum sIL-2Rα level, consequently considered as a signal for lymphocyte activation. Nonetheless, host immune response can also be negatively modulated by sIL-2R (16). In fact, an adequate balance of lymphocyte activation is critical in maintaining homeostasis between responses to foreign antigens and tolerance to self-antigens (42, 43). Elegant in vitro studies of lymphocyte inactivation further support such concept by showing that the profound defect of cellular immunity in patients with advanced cancer may be attributable to the serum factor from cancer patients (44, 45). In addition to activated lymphocytes, increased IL-2Rα gene expression can be found in many different types of human cancer cells (18–31, 46–48). IL-2R expression on cancer cells seems to play a role in tumor growth (46, 47). Although the autocrine tumor proliferation is not observed consistently (48), it provides a focus for future studies to elucidate the mechanism by which expression of sIL-2Rα may be regulated pathophysiological. The impact of sIL-2Rα on tumor progression, nevertheless, remains to be clarified if this is the basis for tumor evasion of host immune surveillance.

Tumor progression is a concerted process including evasion of host immune surveillance, tumor cell proliferation, killing of the immune cell, and the invasion of neighboring tissues as well as distant organs (36). The present study demonstrates that the serum level of sIL-2Rα in ESCC patients is proportional to the tumor burden as well as the disease progression. Expression of IL-2 receptors was detected by IHC in the pathological sections of tumor specimens, and among these specimens, the hybridized products of IL-2Rα mRNA were further identified by ISH. All patients with a high level of serum sIL-2Rα (>1500 pg/ml) were associated with the positive expression of IL-2Rα mRNA. In addition to activated T cells, cancer cells can be an important source of elevated sIL-2Rα in ESCC patients. This elevated sIL-2Rα level can in turn act as a prognostic serum biomarker to assess the aggressiveness of ESCC.

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