Clinica Chimica Acta 415 (2013) 25–30

Contents lists available at SciVerse ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

High expression of interleukin 10 might predict poor prognosis in early stage oral squamous cell carcinoma patients

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ARTICLE INFO

Article history:
Received 8 July 2012
Received in revised form 30 August 2012
Accepted 6 September 2012
Available online 12 September 2012

Keywords:
Interleukin 10
Oral cancer
Survival

ABSTRACT

Background: Interleukin 10 (IL10) plays an important role in immunosuppression and suppression of antitumor immunity. This study examined the IL10 expression of tumor cells and assessed its significance in patients with oral squamous cell carcinoma (OSCC).
Methods: Tumor tissues and adjacent normal tissues were obtained from 325 patients with OSCC and were arranged in a tissue microarray. We examined 325 surgical specimens for associations between IL10 expression in tumor cells and clinical parameters of oral cancer.
Results: High IL10 expression in OSCC patients was significantly associated with male gender ($P<0.001$), smoking ($P=0.015$), alcohol consumption ($P=0.018$), betel quid chewing ($P=0.003$), poor relapse free survival ($P=0.012$), and poor overall survival ($P=0.001$). Patients with high IL10 expression, and particularly early stage OSCC patients, had significantly worse overall survival as defined by the log-rank test ($P=0.014$ for all cases; $P=0.004$ for early stage patients). In early stage patients, high IL10 expression in tumor cells was associated with poor prognosis ($P=0.018$) and a 1.99-fold higher death risk, as determined by Cox regression.
Conclusion: High IL10 expression is significantly associated with aggressive clinical manifestations and might be an independent survival predictor, particularly in early stage OSCC patients.

1. Introduction

Oral squamous cell carcinoma (OSCC) is a significant cause of cancer-related deaths worldwide and is the 4th cause of fatal malignancies in Taiwan [1,2]. Smoking, alcohol consumption, and betel quid chewing are considered to be the main etiologies of OSCC in Taiwan [3]. Most OSCC patients are male; the female oral cancer incidence rate is low in Taiwan [3,4]. The treatments for OSCC include surgery, radiotherapy, and adjuvant chemotherapy, but patient prognosis remains poor [5].

Interleukin 10 (IL10), also known as cytokine synthesis inhibitory factor, is an immunoregulatory cytokine with biological functions of anti-inflammation, immunosuppression, allergy, and anti-agenesis. It operates through the JAK-STAT signaling pathway or by blocking NF-κB nuclear translocation [6,7]. IL10 has also become a therapeutic target in human diseases such as Crohn's disease, rheumatoid arthritis, psoriasis, and some viral infections [7]. The major source of IL10 in normal tissues is macrophages, but IL10 can be also produced by T lymphocytes, B lymphocytes, mast cells, eosinophils, or even keratinocytes [8]. IL10 regulates differentiation and proliferation in immune cells as well as contributing to immune escape of antitumor immunity by down-regulation of MHC class I expression or inhibition T-cell activation and function [9,10].

Increased IL10 expression in either serum or tumor tissues has been noted in many types of cancer, including malignant melanoma, pancreatic cancer, gastric cancer, nasopharyngeal carcinoma, lung cancer, breast cancer, colon cancer, esophageal cancer, hepatocellular carcinoma, and lymphoma [7,11–18] and has been proposed as an indicator of poor prognosis [12,13,17,19]. More specifically, polymorphisms in the IL10 promoter are associated with increased risk of oral, lung, and gastric cancers, lymphoma, and hepatocellular carcinoma [20–24]. In head and neck carcinomas, increased IL10 expression in serum or tumor tissue is associated with poor prognosis [25,26]. However,

Abbreviations: OS, overall survival; OSCC, oral squamous cell carcinoma; RFS, relapse free survival.

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http://dx.doi.org/10.1016/j.cca.2012.09.009

previous investigations involved relatively small study populations and few female patients were included. The prognostic role of tumor-expressed IL10 therefore requires further verification.

In the case of oral cancer, IL10 plays an important role in tumorigenesis, as demonstrated in a few studies that have examined clinical application of IL10 expression in the evaluation of oral cancer. However, in the Taiwanese population, which has a particularly high rate of oral cancer, studies on the role of IL10 are limited. The aim of the present study was therefore to investigate the prognostic value of IL10 in Taiwanese OSCC patients. In addition, we included more female OSCC patients in order to evaluate their clinicopathological features.

2. Materials and methods

2.1. Patients and tissue microarrays

Tissue samples were examined from all 325 OSCC patients with oral cancer who underwent surgical resection at Changhua Christian Hospital between 2000 and 2006. None of the patients received radiotherapy, chemotherapy, or any other treatment prior to surgery. Diagnosis of OSCC was based on histological examination of hematoxylin and eosin-stained tissue sections. We constructed formalin-fixed, paraffin-embedded tissue microarrays composed of 325 OSCC tissue cores, as previously described [27].

The clinical data (sex, age, grade, smoking, alcohol consumption, betel quid chewing, T, N, and M stages, and post-operative adjuvant therapy) and follow-up information (i.e., living or deceased) were obtained from medical records and the cancer registry. This study was approved by the Institutional Review Board and the Ethics Committee of Changhua Christian Hospital (IRB serial number: 111014) and Chung Shan Medical University Hospital (IRB serial number: CS11178).

2.2. Analyses of IL10 expression by immunohistochemistry staining (IHC staining)

Paraffin embedded OSCC tissue sections (4 μm) were placed on coated slides and washed with xylene to remove the paraffin, then rehydrated through serial dilutions of alcohol, followed by washings with phosphate buffered saline, PBS (pH 7.2). After incubation with anti-IL10 antibody (1:5 dilution; sc-8538, Santa Cruz Biotechnology, Santa Cruz, CA) [28] for 60 min at room temperature, the slides were thoroughly washed 3 times with PBS. The conventional streptavidin peroxidase method (LSAB Kit K675; DAKO, Copenhagen, Denmark) was performed for signal development. PBS, instead of primary antibodies, was used as a negative control. Stromal lymphocytes were used as a positive control [7].

The appearance of IL10 staining in the cytoplasm of cancer cells was regarded as positive staining. The staining intensity in the cancerous tissue was graded on a scale from 0 to 3 according to the relative expression intensity and the percentage of cells that showed positive staining was also determined. Immunostaining scores were defined as the cell staining intensity (0 = negative; 1 = weak; 2 = moderate; and 3 = strong) multiplied by the percentage of labeled cancer cells (0–100%), leading to scores that ranged from 0 to 300, as previously described [29]. Expression of IL10 was assessed semiquantitatively by 2 pathologists (Dr. CJ Chen and Dr. KT Yeh), who independently scored coded sections based on the staining score. A final agreement was obtained for each score, even for discrepant immunostaining results.

2.3. Statistical analysis

The correlation of IL10 expression and clinicopathological parameters of OSCC was examined with a Pearson chi-square test. The overall survival (OS) was defined as the time from the initiation of surgery until death. Relapse free survival (RFS) was defined as the time between date of diagnosis and date of local recurrence/distant metastasis. The distribution of OS was estimated using a Kaplan–Meier plot and the log-rank test. The prognostic significance of the variables was evaluated using the Cox regression model and hazard ratios (HR). The variables in the model included gender, stage, T value, N value, M value, tumor grade, and IL10 expression level. The analyses were performed using the Statistical Package for Social Sciences, Version 15.0 (SPSS, Version 15.0, Chicago, IL), and a $P<0.05$ (2-tailed test) was considered statistically significant.

3. Results

3.1. Patient characteristics

In total, 325 patients, including 252 males and 73 females, were analyzed in this retrospective study. The mean age of the patients was 56.2 ± 11.5 years. The histological tumor type of all 325 patients was SCC. In total, 71 patients had stage I tumors, 51 patients had stage II tumors, 31 patients had stage III tumors, and 172 patients had stage IV tumors. Of these tumors, 48 were well differentiated, 271 were moderately differentiated, and 6 were poorly differentiated. The OS time ranged from 0.1 to 9.0 years, with a mean survival time of 3.9 ± 2.8 years and a median survival time of 3.9 years. Adjuvant therapy was administered according to individual considerations. According to the chart records, 314 patients had a history of smoking, 317 patients had alcohol drinking history, and 291 patients had betel quid chewing history. The remaining histories were missing or unrecorded in the charts.

3.2. Higher IL10 expression in OSCC tumor tissue than in adjacent normal tissue

IL10 was expressed in the cytoplasm of cancer cells (Fig. 1). Its expression was diffuse or patchy in the tumor and was also noted in the background lymphocytes. Of the 325 tumors, 315 (96.9%) displayed cytoplasmic IL10 staining (Fig. 1). When the IL10 expression was classified using a 2-tier grading system, namely high (score ≥ 100) and low IL10 expression (score < 100), 143 tumors (44.0%) were classified with high IL10 expression and 182 tumors (56.0%) with low IL10 expression. We also used 21 whole-mount sections to analyze the IL10 expression in the tissues adjacent to the invasive OSCCs. We observed that normal oral epithelium or hyperplastic oral epithelium tended to display negative or faint/barely perceptible cytoplasmic IL10 staining (18 cases, 85.7%) (Fig. 2). Dysplastic oral epithelium tended to show weak cytoplasmic IL10 staining (9 cases, 42.9%) (Fig. 2).

3.3. Relationship between tumor IL10 expression and clinicopathological parameters

When IL10 expression was classified using a 2-tier grading system, high IL10 expression was significantly associated with poorer RFS ($P = 0.012$) and poorer OS ($P = 0.001$) (see Table 1). High IL10 expression was associated with male gender ($P < 0.001$), smoking ($P = 0.015$), alcohol consumption ($P = 0.018$), and betel quid chewing ($P = 0.003$). However, no significant association was found between IL10 expression and patient age, tumor grade, tumor size, lymph node metastasis, distant metastasis, and tumor stage.

3.4. Tumor IL10 expression predicts poor clinical outcome in OSCC patients

Univariate analyses performed using the Cox proportional hazard regression model identified that male gender ($P < 0.001$), advanced clinical stages ($P < 0.001$), larger tumor size ($P < 0.001$), positive lymph node metastasis ($P < 0.001$), poor tumor differentiation ($P = 0.016$), and high IL10 expression score ≥ 100 ($P = 0.024$) correlated with poorer OS (see Table 2). However, in a multivariate analysis model, the OS rate of OSCC