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Original Article

cIAP-2 Expression Increases in Elderly Patients with Squamous Cell Carcinoma of the Head and Neck☆,☆☆

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SUMMARY

Background: Evidence indicates apoptosis deregulation is associated with aging. We correlated expression of cIAP-2, a member of the inhibitor of apoptosis protein family, with survival and age in patients with resected squamous cell carcinoma of head and neck (SCCHN).
Methods: Immunohistochemical (IHC) staining for cIAP-2 and other markers were performed using tissue microarrays from 103 resected SCCHN patients. Expression was grouped according to IHC scores (0–3) and statistical association with clinical–pathological factors was determined.
Results: High expression of cIAP-2 was found to be increased in patients aged 60 or greater. High expression of cIAP-2 and an age ≥60 were significantly associated with worse survival in univariate analysis, but only an age ≥60, and not cIAP-2 expression, was prognostic in multivariate analysis.
Conclusion: High expression of cIAP-2 was found more frequently in elderly SCCHN patients but was not independently associated with survival after adjusting other clinical–pathologic factors.

1. Introduction

Squamous cell carcinoma of head and neck (SCCHN), is the seventh leading cause of cancer death worldwide[1] and refers to a collection of heterogeneous cancers rising from the squamous epithelium of the oral cavity and pharynx. Survival expectations for patients with SCCHN depend on a variety of factors including age. Surgery is traditionally considered curative for early-stage disease in SCCHN. Several clinical trials in older patients with SCCHN have shown that age should not be considered a criterion for disqualifying a patient from surgery[2]. However, in our previous study with surgically resected SCCHN patients, we found patient ages greater than 60 year old is an independent prognostic factor under examination by multivariate analysis, adjusting for other factors such as sex, tumor stage, and clinical–pathological factors[3]. This observation suggested differences in tumor behavior in SCCHN patients 60 years old or older.

SCCHN is characterized by constitutive activation of nuclear factor κB (NF-κB)[4,5]. The DNA-binding activity of NF-κB was demonstrated to be significantly increased with aging in several animal studies[6,7]. Activation of NF-κB induces several anti-apoptotic genes, such as inhibitor of apoptosis (IAP) family members, Bcl-2 family members and the caspase-8 inhibitor[8]. IAP family proteins, such as cIAP-1, survivin, and XIAP, have been shown to have elevated expression levels in many tumors including SCCHN[9–16]. Increased expression levels of cIAP-1, surviving, and XIAP have been correlated with aggressive tumor behaviors[11–26]. However, little is known regarding the clinical relevance of cIAP-2 expression in SCCHN. While, deregulation or inhibition of

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apoptosis contributes to the development and progression of cancer, aging may alter mediation of responses to apoptotic stimuli[27]. Previous investigation of human colon cancer tissues found increased expression of cIAP-2 in tissue from elderly patients (>70 years) compared with younger patients (<70 years)[28]. These observations suggest that the control of inflammation and apoptosis is affected by aging, and may contribute in part to tumor development or progression, in the elderly population.

In the present study, we compare the expression of cIAP-2 in younger (<60 years) and older (≥60 years) patients, and examine the association of cIAP-2 expression levels with survival and other clinical–pathological characteristics.

2. Materials and methods

2.1. Study subjects and tissue samples

The inclusion and exclusion criteria, as well as the baseline characteristics for the patients studied have been described previously[3]. In this study, 15 cases were further excluded from the 118 patients comprising our previous study[3], due to inadequate tumor samples required for further cIAP-2 tissue microarray (TMA) analysis. Briefly, formalin-fixed paraffin-embedded (FFPE) surgical tumor samples from 103 patients diagnosed with SCCHN of the oral cavity, oropharynx, hypopharynx, tonsil, or larynx between 1998 and 2010 who received surgery at Taipei Medical University Wan Fang Hospital were collected. Patients were excluded who died within 2 months after surgery, were lost to follow up, or whose samples were insufficient for TMA analysis. Clinical and pathological information, including demographic data, pathologic TNM stage, and overall survival (OS) were collected by chart review. Patients were monitored until death or April 1, 2012. The study was approved by the institutional review board at Wang Fang Hospital.

2.2. TMA construction, immunohistochemistry, and scoring

For each of the 103 SCCHN samples, both tumor and paired normal mucosal tissues were arrayed in quadruplicate for TMA. Primary antibodies against cIAP-1 and cIAP-2 were obtained from Cell Signaling Technology (Danvers, MA, USA). IHC staining for each antigen was performed on 3-μm paraffin sections, and the expression of each antigen was quantified as previously described[3,29]. A composite score was generated from the staining intensity (0, 1+, 2+, and 3+) and the percentage of positive staining tissue. Since cIAP-1 was strongly overexpressed in over 97% of SCCHN tumor samples, it was not incorporated in subsequent clinico-pathological analysis. Representative examples of each score for cIAP-2 are presented in Figure 1. Tumors with IHC scores of 1+ or 2+ were considered to have low expression, and those with an IHC score of 3+ were considered to have high expression.

2.3. Statistical analysis

Statistical analysis examining expression levels of cIAP-2 was carried out according to patients' clinical–pathological characteristics. Associations between categorical variables were tested using χ^2 or Fisher's exact tests, and correlations were assessed using Spearman's rank correlation test. For survival analysis, the IHC scores for cIAP-2 were grouped as low (0, 1+ and 2+) or high (3+) scoring. Overall survival (OS) rates were analyzed using the Kaplan–Meier method, and compared using the log-rank test for univariate analysis. Subsequently, multivariate analysis using a Cox proportional hazards model was performed to detect independent predictors of survival. All statistical tests were two-sided and *P*-values of less than 0.05 were considered statistically significant.

3. Results

3.1. Expression of cIAP-2 in SCCHN tumors and patient characteristics categorized according to age

The expression of cIAP-2 was mainly localized in the cytoplasm of tumor cells (Figure 1). There were 14 samples out of 103 cases (13.59%) that were scored as negative (IHC score: 0) and 6 out of 103 (5.83%) that exhibited high expression (IHC score: 3) of cIAP-2.

Thirty-three out of 103 studied patients (32.0%) were older than 60. Patient characteristics were listed in Table 1, according to age. In patients aged 60 years or older, significantly more tumors originated from the non-oral cavity (24.2% age ≥60 vs. 2.9% age <60, $P = 0.002$; Fisher's exact test) compared with younger patients. Tumors expressing high levels of cIAP-2 were significantly correlated with patients being 60 years old or older ($P = 0.012$; Fisher's exact test).

3.2. Survival analysis

The average survival time for SCCHN patients was 47.22 months (standard error, SE: 6.29 months) for the age group ≥60 years old and 98.50 months (SE: 8.66 months) for the age group <60 years old. Examination using univariate analysis (Figure 2), demonstrated a statistically significant difference in survival between the groups using the log-rank test ($P = 0.033$). The mean OS for cIAP-2-high expression patients was 37.13 months (SE: 0.45 months), and the mean OS for cIAP-2-negative or low expression patients was 93.94 months (SE: 7.57 months), with a statistically significant difference in survival between the two groups ($P = 0.019$; log-rank test). Univariate analysis for other clinical–pathologic factors such sex, and stage were summarized in Table 2. OS was found to be significantly longer in patients with tumors categorized as earlier stage (stage I and II), and younger patients with age <60. There was no statistical difference in survival between sexes and primary site of the tumors.

To determine whether high expression of cIAP-2 is an independent prognostic factor in SCCHN, multivariate analysis was performed using Cox proportional hazards models. After controlling for age, tumor stage, and primary site of tumor, it was found that the age group ≥60 years and advanced tumor stage (stage III and IV) were independently associated with worse OS having adjusted hazard ratios (HRs) of 2.42 and 4.26, respectively (Table 3). The hazard ratio for high expression of cIAP-2 was 2.17 [95% confidence interval (CI): 0.76–6.15], which was not found to be statistically significant ($P = 0.146$).

4. Discussion

In this retrospective study, we demonstrated that high expression of cIAP-2 was more common in elderly SCCHN patients 60 years old or older compared with younger patients. Univariate analysis indicated both high expression of cIAP-2 and age ≥60 years old were associated with worse prognosis for SCCHN. On examination with multivariate analysis, age ≥60 years old, as well as advanced tumor stage (stage III or IV) remained independent prognostic factors after adjusting for other clinical–pathologic factors. However, high expression of cIAP-2 did not significantly correlate with patient survival (HR: 2.17; 95% CI: 0.76–6.15). Our study was the first to examine expression of cIAP-2 in surgical specimens from SCCHN patients and evaluate correlation of expression with clinical–pathologic factors.

Accumulating evidence has shown that deregulation of apoptosis is associated with the aging process[30]. Evasion of apoptosis has been identified as one of the hallmarks of cancer,