

Co-Expression of XIAP and CIAP1 Play Synergistic Effect on Patient's Prognosis in Head and Neck Cancer

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Abstract

Purpose: To explore the relationship between XIAP and CIAP1 co-expression and the prognosis in HNSCC.

Experimental design: 69 patients were recruited in our study, expression level of XIAP and CIAP1 were examined in all patients, and was correlated with clinical outcomes of the patients.

Results: The patients whose tumors co-express high level of XIAP and CIAP1 presented poorer overall and disease-free survival rates than those whose tumors co-express low level of XIAP and CIAP1 (overall survival P<0.001, disease-free survival P<0.001).

Conclusions: Our results validate that co-expression of XIAP and CIAP1 prompted a worse prognosis.

Keywords: Head and neck; Squamous cell carcinoma; XIAP and CIAP1; Prognosis

Introduction

Squamous cell carcinomas are the sixth most common cancer in developed countries, with 500,000 new cases diagnosed worldwide each year [1]. Despite advances in treatment by surgery, radiation, and chemotherapy, approximately half of patients with HNSCC still die within 5 years, and many of the surviving patients suffer significant impairment in voice, speech, and swallowing as a result of cancer or treatment. Consequently, it is important to identify new molecularly targeted agents that have greater and more selective activity for HNSCC.

Apoptosis resistance enables cancer cells to survive, although exposed in many proapoptotic factors, such as cytotoxic drugs, anoxemia, and radicalization. There are several factors have been found correlated to the carcinoma cell apoptosis resistance, for instance, Bcl-2 and members of the inhibitor of apoptosis [IAP] [2,3]. Recently, the IAPs, as the direct inhibitors of the ultimate effective molecules of apoptosis, have been shown to be important in the process of chemo resistance [4-6]. IAPs represent one set of potent endogenous modulators of apoptosis in mammalian cells. IAPs include a family of intracellular antiapoptotic proteins consisting of eight members: XIAP, cIAP1, cIAP2, survivin, NIAP, Bruce, ML-IAP and ILP-2 [7]. These proteins mediate multiple biological functions that include binding and inhibiting caspases, regulating the cell cycle progression, and modulating receptor-mediated signal transduction [6].

XIAP and CIAP1 were the most potent caspase inhibitor in the IAP family: they bind to and inhibits active caspases 3, 7 and 9, and additionally ubiquitinates them [8-10]. XIAP overexpression in tumor cells has been shown to cause an inhibitory effect on cell death induced by a variety of apoptotic stimuli and induce resistance to chemotherapy [11,12]. Previous study show XIAP and CIAP1 knockdown could enhance tumor cells drug sensitivity [13-25]. Our previous study validates that XIAP RNAi enhances the cisplatin sensitivity of CAL27 and HN13 cells and XIAP expression correlated with poor prognosis in advanced HNSCC patients [26].

There have been no reports about the clinicopathologic significance of XIAP and CIAP1 co-express in HNSCC. Therefore, the aim of our study was to investigate co-expression of XIAP and CIAP1 in HNSCC.

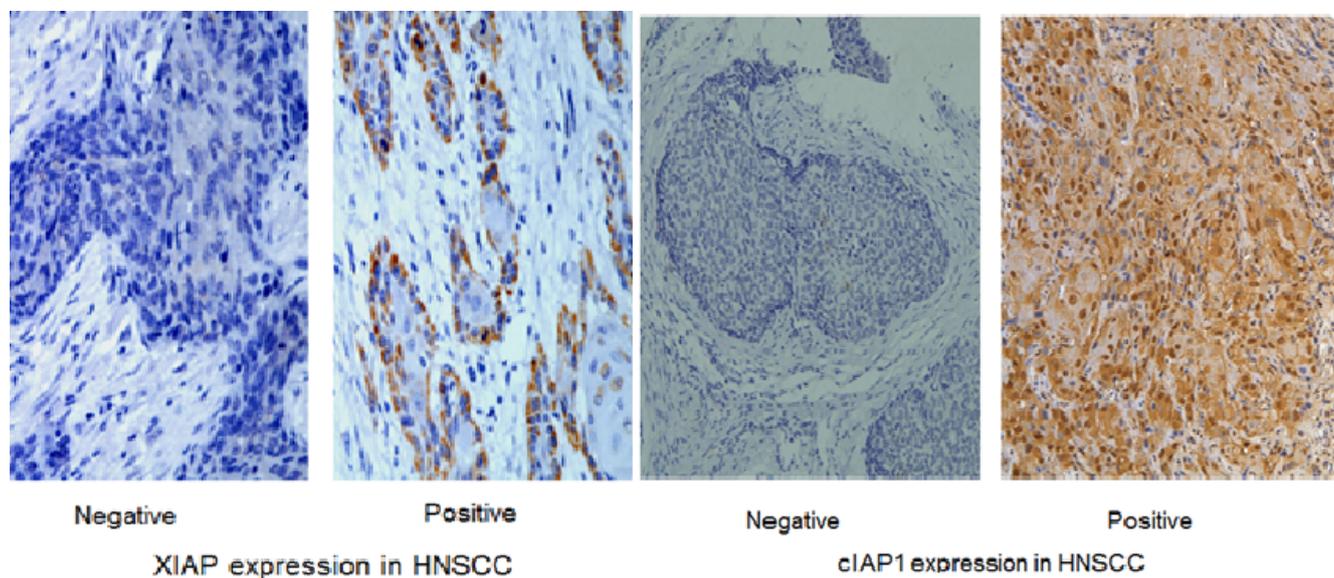
Results

Co-expression of XIAP and CIAP1 predict a worse prognosis in HNSCC

Our previous study validated that XIAP was correlated with chemo response and prognosis in HNSCC. CIAP1 is another common member of IAP. So, we want to know whether Co-expression of XIAP and CIAP1 play synergistic effect for patient's prognosis. Next, we examined XIAP and CIAP1 protein level in 69 patients by IHC. Both were mainly localized in the cytoplasm of tumor cells, with highly variable positive rate from 1% to 90% (Figures 1 and 2). To determine whether XIAP and CIAP1 co-expression can be used to predict patients' clinical outcome in HNSCC, we compared patients' XIAP and CIAP1 co-expression status with overall and disease-free survival

durations. We found that patients with whose tumors co-express high levels of XIAP and CIAP1 had a shorter overall and disease-free survival than did those whose tumors co-express low levels of XIAP and CIAP1 in cancer tissues. The difference in overall and disease-free

survival were statistically significant (overall survival $P < 0.001$ disease-free survival $P < 0.001$) (Figure 3). These results indicate XIAP and CIAP1 play synergistic effect on patient's prognosis, lead to a worse prognosis than XIAP.



Figures 1 and 2: IHC staining of XIAP and cIAP1 in HNSCC. Negative control with PBS instead of first antibody.

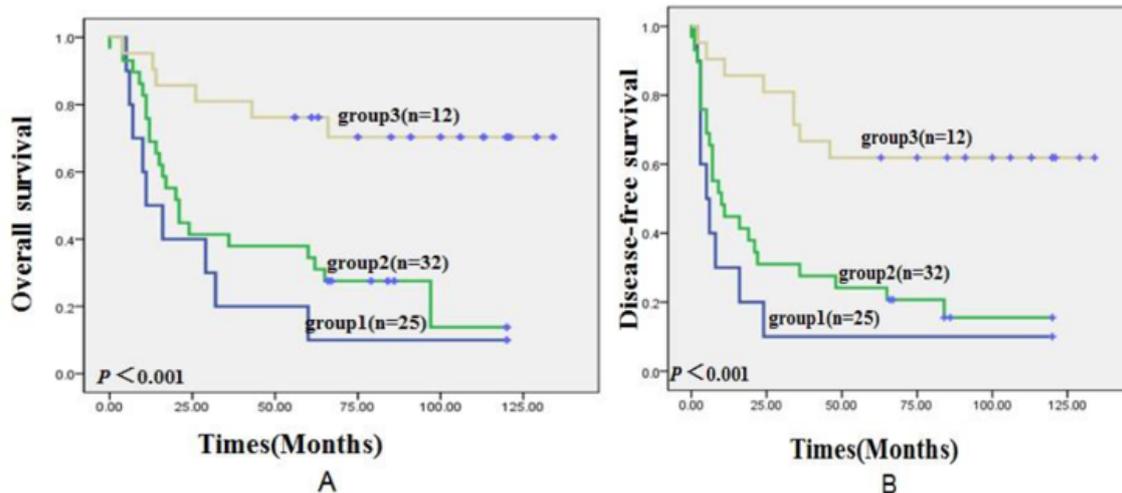


Figure 3: XIAP and CIAP1 co-expression with survival A: co-expression and OS B: co-expression and DFS. group1, high XIAP/high CIAP1; group2, high XIAP/low CIAP1 or low XIAP/high CIAP1; group3, low XIAP/low CIAP1.

Discussion

Resistance to apoptotic stimuli is a hallmark feature of various cancers. One of the mechanisms through which tumor cells are believed to acquire resistance to apoptosis is by overexpression of inhibitor of apoptosis proteins (IAPs) [16]. XIAP and CIAP1 were the most common members of IAP, XIAP is one of the best characterized member of the IAP family in terms of its potent caspase inhibitory

mechanisms and is considered as the prototype of the IAP protein family [11]. CIAP1 can directly inhibit the activity of caspase-3. It has been reported that high levels of XIAP and CIAP1 expression could induce chemo-resistance and radio-resistance of human cancers [17,18]. Thus, XIAP and CIAP1 have been postulated to contribute to the development of some tumors [12].

The positive correlation between the increasing XIAP expression and the poor prognosis such as renal cell carcinoma, colorectal carcinoma, and osteosarcomas [21-23]. Takao [24] have been reported that CIAP1 overexpression correlated with lymph node status and prognosis in HNSCC. Our previous report confirmed the expression of XIAP was associated with drug resistance and poor prognosis [25]. In this study, we examined the XIAP and CIAP1 expression level in 69 patients, find co-expression of XIAP and CIAP1 imply a worse prognosis than their respective expression. This further confirms that chemotherapy resistance is a complex process involving more than one factor.

This study was a retrospective case-control study and had some limitations. In the present study, we chose IHC to evaluate XIAP and CIAP1 expression instead of some quantitative methods primary because of the unavailability of fresh biopsy tissues.

In a word, our data co-expression of XIAP and CIAP1 predicts a worse prognosis with HNSCC. Consequently, XIAP and CIAP1 co-express may be an independent predictor of prognosis for HNSCC.

Materials and Methods

Patients and tumor specimens

69 patients were recruited in our study, they have accepted radical tumor resection at the Department of Oral and Maxillofacial Surgery, Ninth People's Hospital, Shanghai Jiao Tong University from January 1999 to December 2004. Patients' clinicopathologic information is presented in Table 1.

| Variable | No. of patients | % |
|--------------------|-----------------|----|
| Gender | | |
| Male | 53 | 77 |
| Female | 16 | 23 |
| Age | | |
| <60 | 35 | 51 |
| ≥60 | 34 | 49 |
| cTNM stage | | |
| Stage 3 | 24 | 35 |
| Stage 4 | 45 | 65 |
| Pathologic grade | | |
| Stage 1 | 58 | 84 |
| Stage 2 | 9 | 13 |
| Stage 3 | 2 | 3 |
| Smoking history | | |
| Smoker | 31 | 45 |
| Nonsmoker | 38 | 55 |
| Alcohol history | | |
| Drinker | 23 | 33 |
| Nondrinker | 46 | 67 |
| Site | | |
| Tongue | 24 | 35 |
| Gingiva | 16 | 23 |
| Buccal mucosa | 11 | 16 |
| Floor of the mouth | 7 | 10 |
| Oropharynx | 7 | 10 |
| Hard palate | 2 | 3 |

| | | |
|---------------|---|---|
| Nasal sinuses | 2 | 3 |
|---------------|---|---|

Table 1: Clinical characteristics of the patients who participated in study (N=69).

Immunohistochemistry

All patients tissue paraffin blocks were cut into 5 μm sections for standard immunohistochemical staining (IHC). After heat-induced antigen retrieval, slides were incubated with polyclonal mouse anti-human XIAP (BD, USA) at a dilution of 1:100 rabbit anti-human cIAP1 (Santa cruz, USA) at a dilution of 1:500 at 4°C overnight respectively. The omission of the primary antibody served as negative control. Bound antibody was detected by a Super Sensitive IHC Detection System (BioGenex, USA), according to the manufacturer's protocol. The sections were visualized with diaminobenzidine tetrahydrochloride (Sigma, USA) solution and counterstained with Harris hematoxylin. The staining result was determined by counting 1000 tumor cells in three 100x magnification fields by two independent pathologists and further classified as low expression (the percentage of positive rate <25%) and high expression (the percentage of positive rate ≥25%).

Statistical Analysis

The SPSS 17.0 software package was used for statistical analysis. We estimated survival and time-to-progression curves using the Kaplan-Meier method and compared them using a two-sided log-rank test. Differences of P<0.05 were considered statistically significant.

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Author contributions

Conceived and designed the experiment: XHY LL JXQ. Performed the experiments: XHY JXQ YJH JHX. Analyzed the data: XHY LL JXQ. Contributed reagents/materials/analysis tools: PZ. Wrote the paper: XHY LL.

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