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ORIGINAL ARTICLE

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

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Abstract

To explore the influence of chemotherapy on prognosis of Head and Neck Squamous Cell Carcinoma (HNSCC) and the relationship between XIAP and CIAP1 co-expression and the prognosis in HNSCC. 129 patients were recruited in our study, they were divided into two groups, neoadjuvant group ($n = 60$) and non-neoadjuvant group ($n = 69$). Expression level of XIAP and CIAP1 were examed in neoadjuvant group, and was correlated with clinical outcomes of the patients. The unselected patients were not benefit from neoadjuvant chemotherapy**.** Moreover, 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 $P < 0.001$). Our results validate that individual chemotherapy is important for HNSCC, and 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.

Cisplatin is one of the most common anti-cancer agents, and it is widely used in combined chemotherapy of advanced HNSCC. One of its pharmacological mechanisms is to induce caspase-3 activation and apoptosis [2]. Cisplatin induced apoptosis may be one of the most important molecular mechanism of anti-cancer effect of this agent, and the Bcl-2 induced reduction of apoptosis has already been associated with cisplatin resistance in a squamous cell carcinoma cell line A431 [3].

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) [4, 5]. Recently, the IAPs, as the direct inhibitors of the ultimate effective molecules of apoptosis, have been shown to be important in the process of chemoresistance [6–8]. IAPs represent one set of potent endogenous modulators of apoptosis in mammalian cells. IAPs include a family of intracellular antiapopototic proteins consisting of eight members: XIAP, cIAP1, cIAP2, survivin, NIAP, Bruce, ML-IAP and ILP-2 [9]. These proteins mediate multiple biological functions that include binding and inhibiting caspases,

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regulating the cell cycle progression, and modulating receptor-mediated signal transduction [8].

XIAP and CIAP1 were the most potent caspase inhibitor in the IAP family: they binds to and inhibits active caspases 3, 7and 9, and additionally ubiquitinates them [10–12]. 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 [13, 14]. Previous study validated XIAP and CIAP1 knockdown could enhance tumor cells drug sensitivity [15–17]. Our team also showed that XIAP RNAi enhances the cisplatin sensitivity of CAL27 and HN13 cells and XIAP expression correlated with poor prognosis in advanced HNSCC patients who accept chemotherapy [18].

However, the exact mechanism of XIAP and CIAP1 expression in HNSCC drug resistance is not clear to date. Moreover, 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.

Materials and Methods

Patients and Tumor Specimens

129 patients were recruited in our study, they were divided into two groups, neoadjuvant group ($n = 60$) and non-neoadjuvant group ($n = 69$). Neoadjuvant group (n = 60) have accepted cisplatin-based neoadjuvant chemotherapy followed by radical tumor resection within two to three weeks of completing chemotherapy at the Department of Oral and Maxillofacial Surgery, Ninth People's Hospital, Shanghai JiaoTong University from January 1999 to December 2004. Non-neoadjuvant group ($n = 69$) have accepted radical tumor resection and radiation therapy. Patients' clinicopathologic information is presented in Table 1.

Table 1 Clinical characteristics of the patients who participated in study ($N = 129$)

	Neoadjuvant		Non-neoadjuvant	
	Group ($n = 60$)		Group ($n = 69$)	
Variable	No. of patients	%	No. of patients	%
Gender				
Male	39	65	53	77
Female	21	35	16	23
Age				
< 60	37	62	35	51
≥60	23	38	34	49
cTNM stage				
III	21	35	24	35
IV	39	65	45	65
Pathologic grade				
I	40	67	58	84
II	17	28	9	13
III	3	5	2	3

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 at 4 °C overnight. 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 diaminobenzidinetetrahydrochloride (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.

Results

Unselected Patients cannot Benefit from New Adjuvant Chemotherapy

During follow-up period, 77 (60%) of the 129 patients had died (neoadjuvant group: 37 cases and non-neoadjuvant group:40 cases). The DFS rates in neoadjuvant group and non-neoadjuvant group were 32% and 38%, and that the OS rates were 38% and.

42%, respectively. Compared to non-neoadjuvant group, it appears that the addition of chemotherapy did not improve the long-term survival of the patients in the whole neoadjuvant group (Figs. 1 and 2), and there was no significant difference in DFS ($P = 0.524$) and OS ($P = 0.722$) in the two groups. All of these confirmed the importance of selective chemotherapy.

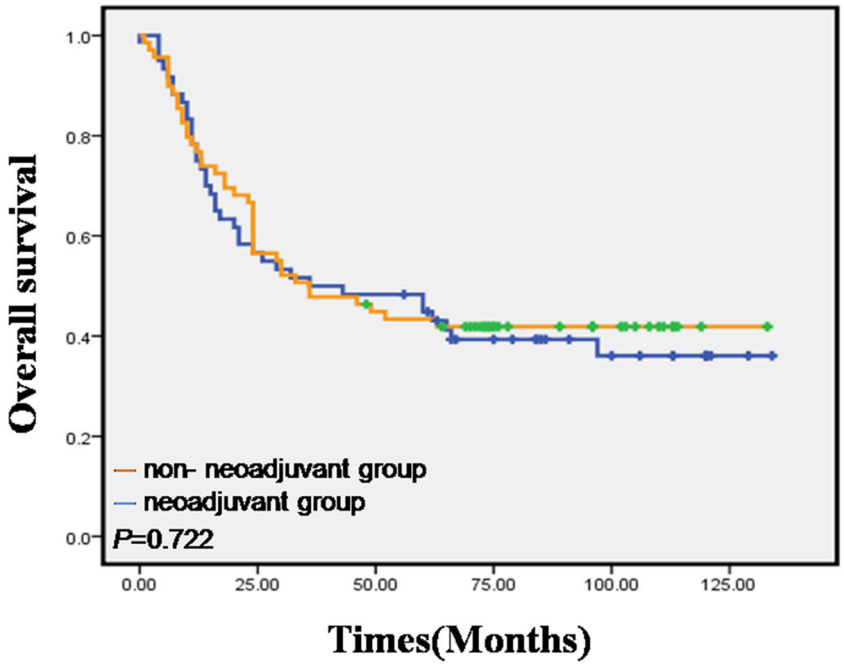

Fig. 1 Overall survival in two groups

Co-expression of XIAP and CIAP1 Predict a Worse Prognosis in HNSCC

Our previous study validated that XIAP was correlated with chemoresponse and prognosis in HNSCC. CIAP1 is another common member of IAP. Therefore, we would like to know the effect of co-expression of XIAP and cIAP1 on patients' prognosis. Next, we examined the expression level of XIAP and CIAP1 in the neoadjuvant group by IHC ($N = 60$). Both of them were mainly localized in the cytoplasm of tumor cells, with highly variable positive rate from 1%–90% (Fig. 3). 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

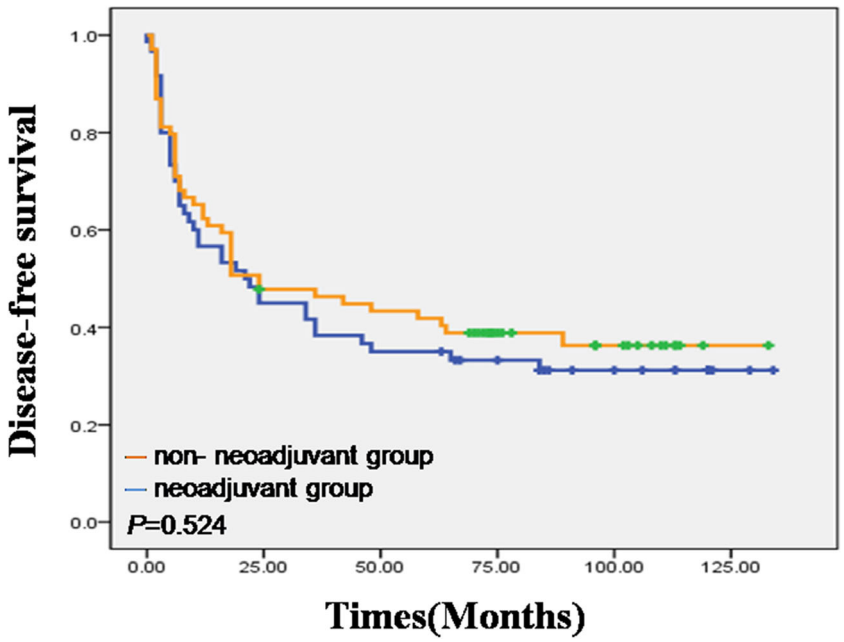

Fig. 2 Disease free survival in two groups

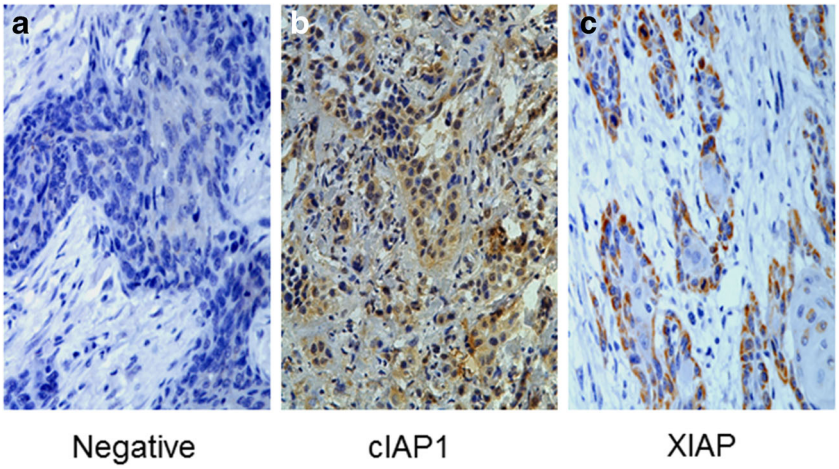

Fig. 3 IHC staining of XIAP and CIAP1 in advanced HNSCC. **a** negative control with PBS instead of first antibody; **b** CIAP1 expression; **c** XIAP expression

survival $P < 0.001$ disease-free survival $P < 0.001$) (Figs. 4 and 5). These results indicate XIAP and CIAP1 play synergistic effect on patient's prognosis, lead to a worse prognosis than XIAP.

Discussion

Chemotherapy is one of the most important treatment methods for malignant tumors. Neoadjuvant chemotherapy, including many regimens, has been investigated in head and neck cancer, however, the results remain inconclusive, if not negative [19]. In our study, we find that the unselected patients cannot benefit from new adjuvant chemotherapy, it did not improve the survival of unselected patients with HNSCC. Drug resistance is the main factor for the failure of chemotherapy. All of these validated the importance of selective chemotherapy.

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) [20]. 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 [13]. 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 [21, 22]. Thus, XIAP and CIAP1 have been postulated to contribute to the development of some tumors [14].

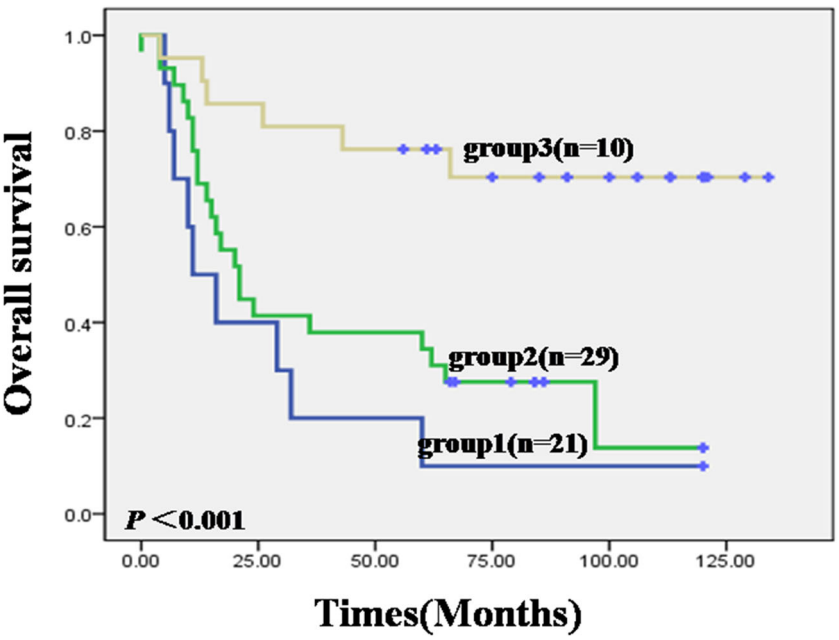

Fig. 4 XIAP and CIAP1 co-expression with overall survival group1, high XIAP/ high CIAP1; group2, high XIAP/ low CIAP1 or low XIAP/high CIAP1; group3, low XIAP/ low CIAP1

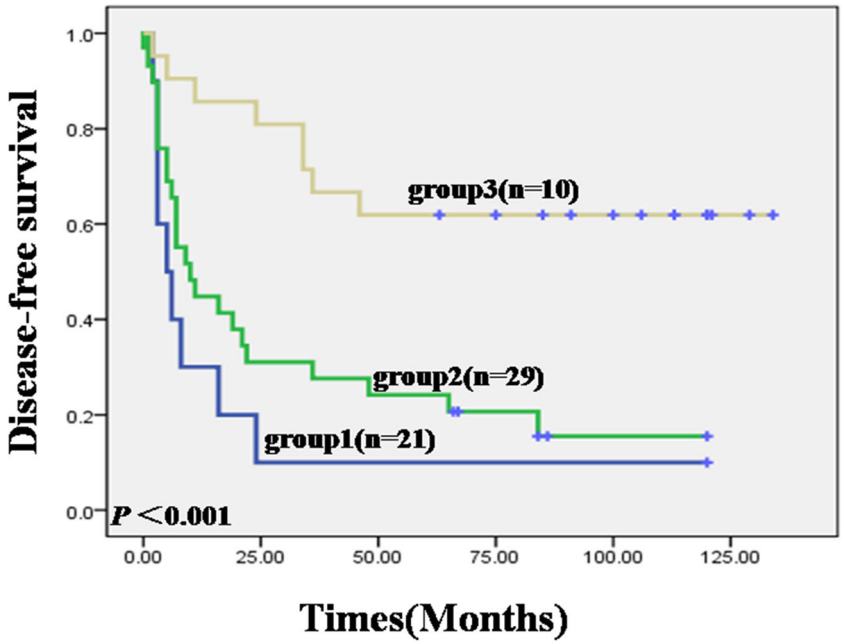

Fig. 5 XIAP and CIAP1 co-expression with Disease free survival group1, high XIAP/ high CIAP1; group2, high XIAP/ low CIAP1 or low XIAP/high CIAP1; group3, low XIAP/ low CIAP1

In many cancers, increased expression of XIAP is positively associated with poor prognosis in patients, such as renal cell carcinoma, colorectal carcinoma and osteosarcomas [23–25]. Takao [26] has 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 [18]. In neoadjuvant group ($n = 60$), 34 had complete response(CR) and partial response(PR), with the overall survival rates of 53%; 26 had stable disease(SD) and progression disease(PD), with the overall survival rates of 19%, the relationship between chemoresponse and overall survival were positive correlation. All of these further validated that individualized chemotherapy is important for HNSCC, not all patients suitable for chemotherapy. In this study, we examed the XIAP and CIAP1 expression level in neoadjuvant group, found that co-expression of XIAP and CIAP1 imply a worse prognosis than their respective expression. This further confirms that chemotherapy resistance is a comlex process involving more than one factor.

Previous studies have shown that high doses cisplatin was capable of inhibiting XIAP and CIAP1 expression in ovarian cancer cells, thyroid cancer and OSCC [17, 27, 28], and overexpression XIAP could inhibit the cisplatin-induced apoptosis in OSCC carcinoma cell lines [28]. The mechanism of XIAP resistance to chemotherapy deserves further study. Downregulation of XIAP and CIAP1 by RNAi and antisense approaches sensitizes cancer cells to chemotherapeutics, including lung cancer, prostate cancer, ovarian cancer, thyroid cancer and pancreatic cancer [7, 15–17, 21]. In our previous studies, we also demonstrated that XIAP RNAi can increase cisplatin sensitivity in HNSCC cells. Meanwhile, XIAP molecular antagonists have been designed, such as SMAC, HtrA2/Omi [29], all of these show great promise for cancer therapy, we believe that there will be more new small molecular antagonists in the near future.

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 suggest that the unselected patients cannot benefit from new adjuvant chemotherapy, and co-expression of XIAP and CIAP1 predicts a worse prognosis with HNSCC. Consequently, XIAP and CIAP1 co-express may be an independent predictors of selective chemotherapy and prognosis for HNSCC.

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Author Contributions Conceived and designed the experiment: XHY LL PZ. Performed the experiments: XHY YJH. Analyzed the data: XHY PZ. Contributed reagents/materials/Analysis tools: PZ QGH. Wrote the paper XHY LL.

Compliance with Ethical Standards

Competing Interests The authors have declared that no competing interests exist.

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Conflict of Interest All authors have approved that there are no potential conflicts of interest.

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