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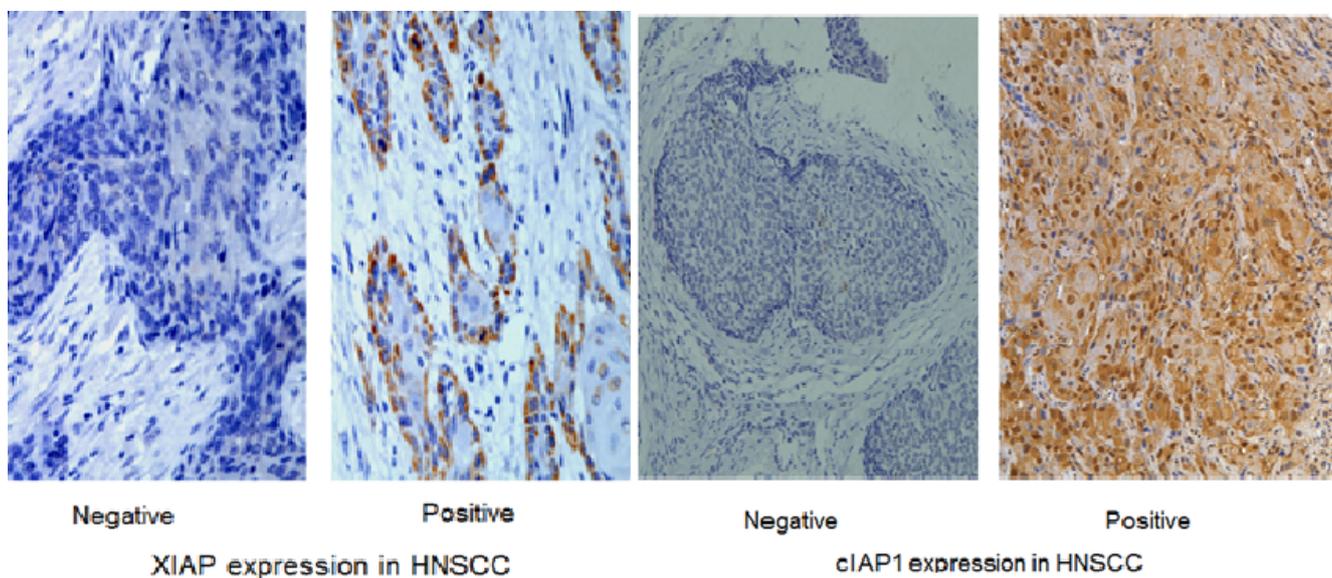
Figures 1 and 2: IHC staining of XIAP and cIAP1 in HNSCC. Negative control with PBS instead of first antibody.
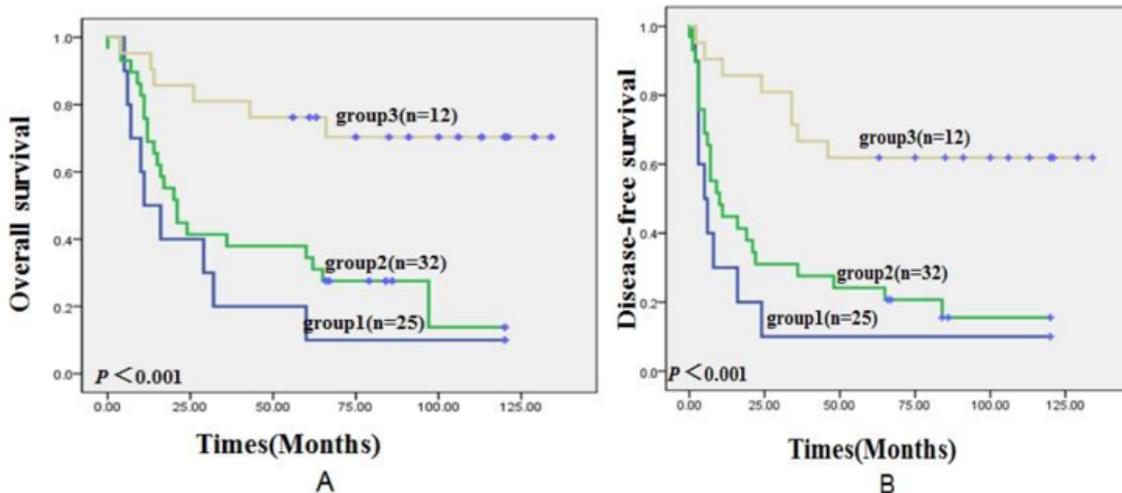

Figure 3: XIAP and CIAP1 co-expression with survival A: co-expression and OS B: co-expression and DFS. group1, high XIAP/high CIAP1; group2, high XIAP/low CIAP1 or low XIAP/high CIAP1; group3, low XIAP/low CIAP1.

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 1:100rabbit 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 diamino benzidine 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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