Expression of cIAP1, a Target for 11q22 Amplification, Correlates with Resistance of Cervical Cancers to Radiotherapy

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Abstract

Inhibition of, or increased resistance to, apoptosis is a common property of cancer cells. This means that a constitutive activation of antia apoptotic molecules via genetic or epigenetic mechanisms, including gene amplification, may well be involved in carcinogenesis. Recently we reported that cIAP1, an inhibitor of apoptosis, is overexpressed through 11q22 amplification in cell lines derived from esophageal squamous cell carcinomas and is associated with resistance of esophageal squamous cell carcinomas to drug-induced apoptosis (I. Imoto et al. Cancer Res., 61: 6629–6634, 2001). Because amplification of 11q22 has been implicated in other malignancies also, including cervical squamous cell carcinomas (CSCCs), we attempted to correlate amplification and overexpression of cIAP1 with radiation sensitivity in CSCC-derived cell lines and primary CSCC tumors. In the nine cell lines we examined, two showed amplification and consistent overexpression of cIAP1, as well as significant resistance to radiation-induced cell death as compared with lines showing no cIAP1 amplification. Immunohistochemical analysis of 70 primary CSCCs from patients treated only with radiotherapy demonstrated that both overall survival and local recurrence-free survival was significantly poorer among patients with tumors showing high levels of nuclear cIAP1 staining than among patients whose tumors revealed little or no nuclear cIAP1. Multivariate analysis showed nuclear cIAP1 staining to be an independent predictive factor for local recurrence-free survival after radiotherapy among patients with CSCC. These findings demonstrate that cIAP1 may play an important role in the development/progression of this disease and that cIAP1 could be a novel predictive marker for resistance to radiotherapy in individual CSCC patients.

Introduction

Cervical cancer is one of the most common tumors affecting women worldwide in terms of both incidence and mortality, with an estimated 500,000 new cases diagnosed annually (1). Radiotherapy, which inhibits growth and causes apoptosis, is the most important nonsurgical treatment for all stages of this disease. However, the response of individual tumors to radiotherapy varies widely. Therefore, early prediction of tumor responsiveness to radiation would allow clinicians to identify patients who should be considered for alternative methods of treatment (radioresistant tumors) or given lower doses of irradiation (radiosensitive tumors). Although tumor stage and tumor size may serve successfully as markers for responsiveness to radiotherapy, they are not likely to account fully for the observed variability (2). Additional indicators, including genetic alterations, are needed to predict more accurately the outcome of radiotherapy for an individual patient.

Multiple genetic alterations have been identified in cervical cancers. They may be linked in a sequence of events that parallels malignant progression. Cervical cancer often arises in women infected with specific types of HPV* (3). However, HPV infection, although apparently necessary, is not sufficient for malignant transformation, implying that other molecular alterations, most likely involving activation of oncogenes and/or inactivation of tumor suppressor genes, must accompany the HPV infection before full development and progression of invasive cervical cancer can take place. Amplification of DNA in certain chromosomal regions is one of the mechanisms for activating genes that are critical in the development and progression of human cancers (4), and numerous oncogenes or other cancer-related genes have been identified in amplified regions. In cervical cancers, especially in CSCCs, proto-oncogenes such as EGFR (7p12), MYC (8q24), ERBB2 (17q11.2–12), CCND1 (11q13), and HRAS (11p15.5) are often activated by amplification (5–9), and some of them are clearly associated with malignant phenotypes (8, 9). However, by themselves these known oncogenes cannot fully account for all of the genetic materials present in amplified regions or for malignant phenotypes, including radioresistant ones. Cumulative results of recent studies involving comparative genomic hybridization indicate that additional amplification targets in CSCC are yet to be identified (10–12). To gain new insights into the molecular pathogenesis of cervical cancer and to establish diagnostic markers and therapeutic targets, additional targets of amplification must be identified.

We recently identified cIAP1, a gene that encodes an antia apoptotic molecule in the IAP family, as a potential target within the 11q22 amplicon observed with some frequency in ESCCs, and provided evidence that ESC cell lines overexpressing this gene were resistant to apoptosis induced by chemotherapeutic reagents (13). An amplicon spanning the same region at 11q22 has been noted, although infrequently, in CSCCs as well (10–12); and it is reasonable to suppose that deregulated apoptosis might play an important role in the development, progression, or responsiveness to radiotherapy of CSCCs (14–18). That is, if cIAP1 is overexpressed via amplification in some CSCCs, it may be associated with an unfavorable prognosis after radiotherapy and/or chemotherapy. However, relationships between malignant phenotype and copy-number or expression status of cIAP1, as well as the functional role of this gene in CSCCs, have not been investigated sufficiently (19). For the study presented here, we first screened cervical cancer cell lines for amplification and overexpress-

*The abbreviations used are: HPV, human papillomavirus; CSCC, cervical squamous cell carcinoma; ESCC, esophageal squamous cell carcinoma; HDR, high dose rate; FISH, fluorescence in situ hybridization; BAC, bacterial artificial chromosome; FIC, fluorescent immunocytochemistry; IHC, immunohistochemistry; IAP, inhibitor of apoptosis; XIAP, x-linked inhibitor of apoptosis.
sion of cIAP1 and attempted to correlate the results with radioresis-
tance. Then we examined the significance of cIAP1 expression as a
predictive marker for resistance to radiotherapy in primary cases of
this disease.

Materials and Methods

Cell Lines and Primary Tumors of CSCCs. All nine human cell lines used in
this study (SKG-I, SKG-II, SKG-IIIa, SKG-IIIb, P98AH2, SHIA, Ca-Ski,
ME180, and HT-3) were established from squamous cell carcinomas. The cells
were maintained in RPMI 1640 supplemented with 10% fetal bovine serum
and 100 units/ml penicillin/100 μg/ml streptomycin.

Samples of primary CSCCs from 97 unrelated patients were provided by the
National Cancer Center Hospital and Kyouondo Hospital, with written consent
from each patient in formal style and after approval by the local ethics
committee. Tumors were classified according to the International Federation
of Gynecology and Obstetrics system. Among them, the frozen tissue specimens
for initial dot blot analysis were obtained by punch biopsy from 27 unselected
patients (stage Ib, 5; stage IIa, 3; stage IIb 1; and stage IIb, 18). The paraffin-embedded
tissue specimens for immunohistochemical analysis were obtained by punch biopsy from the remaining 70 patients before radiotherapy
(stage Ib, 6; stage IIA, 12; stage IIb, 8; stage IIIA, 1; stage IIIB, 32; stage IVa, 8;
and stage IVb, 3). These 70 patients were diagnosed and treated only with
radiation between 1991 and 1996. Treatment techniques of radiotherapy were
designed based on “General Rules for Clinical and Pathological study of
Uterine Cervical Cancer in Japan” (20) with some modifications (21). Briefly,
the external radiation was delivered with 10-MV X-rays from a linear acceler-
ator at a total dose of 50 Gy to whole pelvis via anterior-posterior and
posterior-anterior portals. A daily dose of 2 Gy was given 5 days weekly.
Parametrical boosts using simple rectangular midline shielding blocks (4 cm
wide at the midplane) were individualized according to stage to avoid over-
dosage to the bladder and rectum. For stage I patients, a central shielding was
used during the entire course of irradiation. A central shielding was set after 20
Gy for stage II patients and after 30 Gy for stage III and IV patients. For
intravaginal irradiation, HDR brachytherapy using the remote after-loading
system was performed. Patients were treated once weekly with a dose of 6 Gy
to point A. Stage I patients typically received five sessions of HDR brachy-
therapy to a total dose of 30 Gy. For stage II patients, a central shield was
placed after 20 Gy so that from the third week onward, patients had four HDR
brachytherapy sessions once weekly to a total brachytherapy dose of 24 Gy.
For stage III and IV patients, a central shield was placed after 30 Gy, so that
patients underwent three HDR brachytherapy sessions from the fourth week
and the dose delivered was 18 Gy. The relationship between immunohisto-
chemical findings and overall survival or local recurrence-free survival, cal-
culated from the date of the last radiation treatment to the date of the latest
follow-up visit or death, was analyzed for all 70 patients. The median follow-
up periods were 44.8 months (range, 3.3–77.2 months) for 47 patients
who did not suffer recurrence, and 49.4 months (range, 1.3–91.9 months)
for 42 patients who are alive at the time of this writing.

FISH. Metaphase chromosome slides were prepared for FISH experiments as
described previously (13, 22). BAC RP11–864G05 and 241D13 that contain
cIAP1 and ATM (ataxia telangiectasia mutated), respectively, were labeled
by nick-translation with biotin-16-dUTP and digoxigenin-11-dUTP (Roche Diag-
nostics, Tokyo, Japan), respectively. The copy-number and molecular organi-
zation of the chromosomal regions of interest were assessed according to
hybridization patterns observed on both metaphase and interphase chromo-
somes.

Southern-, Dot-, and Northern-blot Hybridizations. These analyses
were carried out as described elsewhere (13). For Southern blots, 10 μg of
genomic DNA from each cell line or from normal lymphocytes were digested with EcoRI, separated in a 0.8% agarose gel, and transferred onto a nylon
membrane. For dot blots, 2 μg of DNA from the 27 primary tumors or
nontumorous myometrial tissues were denatured with 0.4 N NaOH and trans-
ferrred to a nylon membrane. For Northern blots, 10 μg of total RNA extracted
from each cell line were electrophoresed in 1.0% agarose/0.67 M formaldehyde
gel, then transferred to a positively charged nylon membrane. Membranes were
hybridized with [α-32P]dCTP-labeled cIAP1 cDNA (Integrated Molecular
Analysis of Genome and their Expression clone 3908352) or glyceraldehyde-
3-phosphate dehydrogenase probes, washed, and then exposed as described
previously (13).

FIC. Indirect FIC was performed as described elsewhere (13, 23). In brief,
cultured cells were fixed with acetone/methanol (1:1 v/v), blocked with anti-
body dilution buffer (1% BSA in PBS), and then incubated with 1 μg/ml
anti-human cIAP1 polyclonal antibody (H-83) for 1 h at room temperature.
Normal rabbit serum served as a negative control for the first antibody.
Binding was detected by incubation with FITC-conjugated goat antirabbit IgG
diluted 1:200; ICN Pharmaceuticals, Aurora, OH). The cells were counter-
stained with 4',6-diamidino-2-phenylindole.

Radiation Survival in Cervical Cancer Cell Lines. Clonogenic survival
was determined at radiation doses from 2 to 6 Gy (0.76 Gy/min). Cells from
logarithmically grown cultures were removed by trypsinization, counted, irra-
diated with a Siemens Stabillipan-2 X-ray Unit (Siemens, Erlangen, Germany),
and then plated in triplicate. Colonies were allowed to grow for 14 days before
being stained with 0.05% crystal violet, and those with >50 cells were
counted. The survival rate at a given dose was defined as follows: (number of
colonies formed)/number of cells plated × (plating efficiency).

IHC and Scoring Method. Indirect IHC was performed on formalin-fixed,
paraffin-embedded tissue sections, as described elsewhere (13, 24). The
sections were dewaxed and rehydrated in graded concentrations of ethanol.
Antigens were retrieved by microwave pretreatment in 10 nm citrate buffer
(pH 6.0) for 10 min. After cooling, the sections were treated with 3% hydrogen
peroxide to block endogenous peroxidase, then reacted overnight at 4°C with
0.67 μg/ml anti-human cIAP1 polyclonal antibody (H-83) or normal rabbit
antibody. The sections were rinsed, incubated with rabbit EnVision+ peroxidase
(Dako, Carpinteria, CA), stained with 0.05% hydrogen peroxide and 3,3'-
diaminobenzidine, and counterstained with hematoxylin. Two formalin-fixed
ESC cell lines with known overexpression of cIAP1 (13) were used as positive
controls and also as negative controls where the primary antibody was omitted.

Each section was examined at ×400 magnification. Because the levels of
immunoreactive cIAP1 in cytoplasm and the nucleus are independent (19, 25),
we evaluated them separately. Nuclear cIAP1 distribution was scored as 0 for
no staining, 1+ for <1% of cells staining positive for cIAP1, and 2+ for >1% of
cells staining positive for cIAP1. Cytoplasmic cIAP1 distribution was
scored as 0 for no staining, 1+ for a light and foamy staining pattern, and 2+
for a heavy and diffuse staining pattern. The observer who assessed all staining
results was blinded to the clinical outcomes of the patients.

Statistical Analysis. Possible correlations between variables of the analy-
zed tumor samples were tested by the χ2/Fisher’s exact test. The Mann-
Whitney U test was used to compare the mean counts of radiation-survived
colonies among different cell lines. Univariate and multivariate survival anal-
yses were performed using the likelihood ratio test of the stratified Cox
proportional-hazards model. Kaplan-Meier survival plots were constructed,
and log-rank tests were used for comparisons between groups. P < 0.05 was
required for significance.

Results

Amplification and Subsequent Overexpression of cIAP1 in Cell
Lines Derived from CSCCs. The amplification status and expres-
sion levels of cIAP1 in nine cervical cancer cell lines are shown in Fig. 1.
Remarkable amplification of cIAP1 was detected in Ca-Ski and
SKG-II by FISH (Fig. 1A) and on Southern blots (Fig. 1B), and the
cIAP1 transcript was consistently overexpressed in those two cell
lines (Fig. 1C). FIC detected overexpression of cIAP1 at the protein
level, predominantly in the cytoplasm (Fig. 1D), in the same two cell
lines, whereas nuclear distribution of cIAP1 was also observed in
parts of Ca-Ski (Fig. 1D) and SKG-II (data not shown).

Resistance of Cervical Cancer Cell Lines with cIAP1 Amplifi-
cation to Radiation-induced Cell Death. Because we had demon-
strated previously that ESC cell lines with cIAP1 amplification were
resistant to cell death induced by either chemotherapeutic reagents or
radiation (13), we investigated whether the two cervical cancer cell
lines with cIAP1 amplification were also resistant to radiation-induced
cell death. As shown in Fig. 1E, both Ca-Ski and SKG-II showed
greater resistance to radiation-induced cell death than control lines

4861
cIAP1 was not overexpressed (Fig. 1, C and D).

Amplification of cIAP1 in Primary CSCCs. Because cIAP1 was amplified in some of our cervical cancer cell lines, we examined 27 additional primary CSCCs, from which high-quality genomic DNAs were available; these tumors were unrelated to the cell lines or to the 70 cases we analyzed by IHC. To determine whether amplification of cIAP1 had occurred in any of them, we prepared dot-blot, which indicated an increased signal of the cIAP1 gene in 5 of 27 tumors examined (18.5%; Fig. 2).

Immunohistochemical Expression of cIAP1 in Primary CSCCs. Because cervical cancer cell lines with cIAP1 amplification and overexpression featured a radioresistant phenotype, we analyzed the predictive and prognostic value of cIAP1 expression for 70 CSCC patients, all of whom had received only radiotherapy.

As shown in Fig. 3, immunoreactivity for cIAP1 was predominant in cancer cells as opposed to neighboring normal cells, but staining patterns were heterogeneous and diffuse. As reported previously (19, 25), cIAP1 immunoreactivity can be detected in both cytoplasm (Fig. 3A) and nuclei (Fig. 3B) in paraffin-embedded tumor sections. The possibility that this staining pattern was an artifact because of antigen retrieval can be excluded for two reasons. One is that Ferreira et al. (25) reported a similar pattern during IHC analysis of frozen sections of matched cases without antigen retrieval, and the other is that we observed both cytoplasmic and nuclear staining in IF immunohistochemistry of our cervical cancer cell lines (Fig. 1D) and also in preliminary IHC examinations of primary tumors where cIAP1 amplification had been detected by dot-blot analysis (data not shown).

Among the 70 CSCCs we analyzed, 16 (22.9%) showed positive nuclear immunoreactivity of cIAP1 in >1% of cancer cells (N2+), whereas 10 (14.3%) showed <1% immunoreactivity (N1+) and 44...
(62.9%) showed none (N0; Table 1). On the other hand, six tumors
(8.6%) showed heavy and diffuse cytoplasmic staining (C2+); 29
(41.5%) showed light and foamy staining (C1+) and 35 (50%)
showed none (C0). A score of N2+ was more frequently seen in
tumors at advanced clinical stages (III and IV) than in stages I or II.
The same correlative tendency was observed for scores of C2+.
Ages of the patients showed no significant correlation with immunohis-
tochemical status of cIAP1 (Table 1).

Correlation between cIAP1 Expression and Survival Rate after
Radiotherapy. Fig. 4 shows Kaplan-Meier survival curves for all 70
cases of CSCC patients or 44 cases of grade III and IV CSCC patients.
Women whose tumors showed positive nuclear cIAP1 staining in
>1% cancer cells (score N2+) had significantly shorter local recur-
rence-free and overall survival time than did patients with staining in
<1% of cancer cells (N1+) or none (N0) in their tumors (P = 0.0008,
Fig. 4A and P = 0.0276, Fig. 4B, respectively; log-rank test). In
addition, these influences of N2+ nuclear cIAP1 staining on local
recurrence-free and overall survival time remained significant
(P = 0.0105, Fig. 4C and P = 0.0294, Fig. 4D, respectively; log-rank
test) when the outcome of only the stage III and IV patients was
stratified by nuclear cIAP1 staining pattern. Cytoplastic staining
of cIAP1, on the other hand, did not reflect significant differences
in local recurrence-free survival (P = 0.97) or overall survival
(P = 0.38) among patients expressing heavy and diffuse (C2+), light
and foamy (C1+), or no (C0) staining (data not shown). The log-rank
test for the Kaplan-Meier survival curve showed that patients with
advanced clinical stages or who were <55 years old showed signifi-
cantly shorter local recurrence-free and overall survival time than did
those with earlier clinical stages (P = 0.014 and 0.002, respectively)
or who were >55 (P = 0.0031 and P < 0.0001, respectively; data not
shown).

Univariate analysis demonstrated that clinical stage, age, and nu-
nuclear cIAP1 staining all correlated with both local recurrence-free
survival and overall survival (Table 2). When the data were stratified
for multivariate analysis with respect to stage and age, nuclear cIAP1
staining remained significant at P = 0.015 for local recurrence-free
survival. This indicated that nuclear cIAP1 staining is an independent
predictor of local recurrence-free survival after radiotherapy in pa-
patients with CSCC. As for overall survival, both stage and age proved
to be independent prognostic factors (P = 0.0003 and P < 0.0001,
respectively), whereas nuclear cIAP1 staining was of marginal sig-
nificance (P = 0.082).

Discussion

Suppression of apoptosis is believed to contribute to tumorigenesis
by abnormally prolonging cellular life span, enhancing growth factor-
dependent cell survival and resistance to immunobased cytotoxicity,
and allowing cells to miss cell cycle checkpoints that would normally
induce apoptosis (26). Among several molecules implicated in the
deregulation of apoptosis in cancer cells, the product of cIAP1 is
thought to be one of the more important contributors to carcino-
genesis. cIAP1 is expressed in cell lines derived from a variety of cancer
tissues, where its expression level correlates with resistance to apo-
potic cell death induced by chemotherapeutic reagents (27, 28).
Analyses using cDNA microarrays have detected enhanced expres-
sion of cIAP1 also in experimental models of cancer (29, 30); for
example, Dong et al. (29) identified cIAP1 as an overexpressed gene,
related to the nuclear factor κB pathway, in a metastatic model of
murine squamous cell carcinoma. Overexpression of cIAP1, appar-
etly the consequence of an increase in the number of copies of 11q,
was also observed during progression of a human cell line model for
prostate cancer (30).

As for the ant apoptotic function of cIAP1, its ectopic expression in
mammalian cells can inhibit apoptosis induced by a variety of stimuli,
as in the case of other IAP family members (31, 32). Those reports are
compatible with a previous study of ours where we demonstrated that
overexpression of cIAP1 is generated by the 11q22 amplification
mechanism in ESCs and contributes to resistance of affected cells to
the cell death activity induced by chemotherapeutic agents (13) and
radiation (unpublished data). The overall evidence has led us to the
hypothesis that cIAP1 may be a useful biomarker for predicting the
effect of chemotheraphy and/or radiotherapy in patients with various
types of cancers.

To evaluate that hypothesis, we extended our previous study using
CSCC-derived cell lines and radiation-treated primary tumors of
CSCC. The cell lines showed resistance to radiation-induced cell

<table>
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<tr>
<th>Score of cIAP1 expression</th>
<th>Nuclear staining of cIAP1^a</th>
<th>Cytoplasmic staining of cIAP1^a</th>
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<tr>
<td>0 or 1+</td>
<td>0.019</td>
<td>0.019</td>
</tr>
<tr>
<td>2+</td>
<td></td>
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^a Scores of cIAP1 expression were classified as 0, 1+, and 2+, as described in "Materials and Methods."
^b Score 0 or 1+ versus Score 2+. P values are from two-sided tests and were statistically significant when <0.05. Statistically significant values are in boldface type.

Fig. 3. Immunohistochemical staining of cIAP1 protein in primary tumors. A, representative heavy and diffuse cytoplasmic staining in tumor cells (arrowheads). B, representative nuclear staining in tumor cells (arrows). ×200.
death as a result of amplification and overexpression of cIAP1 (Fig. 1). Consistent with this in vitro finding, Kaplan-Meier survival plots demonstrated an inverse correlation between nuclear expression of cIAP1 and overall survival after radiotherapy, as well as local recurrence survival, among patients with CSCC (Fig. 4). Univariate analysis found a significant correlation between advanced clinical stages, patient age of <55, or an N2+ score of nuclear cIAP1 immunoreactivity and both earlier local recurrence and death after radiotherapy (Table 2). Finally, multivariate analysis showed that an N2+ score for nuclear cIAP1 immunoreactivity correlated with a high risk of local recurrence regardless of clinical stage or patient age and that its risk ratio was 2.82 times higher than that for scores of N1 or N0, even after adjustment by stage and patient age (Table 2). The findings indicate that this molecular alteration may be useful as a marker to identify CSCC patients who carry a high risk of local recurrence. Several genetic markers, including HPV infection, loss of heterozygosity on 6p21.2, loss of heterozygosity on 18q21.2, and COX-2, have been identified recently as determinants of prognosis for cervical cancer patients treated with radiotherapy (2, 33). To examine whether these molecular markers and cIAP1 are mutually independent predictors, it will be necessary to compare the expression of cIAP1 and abnormalities of any other genetic markers in more cases of CSCC treated with uniform radiotherapy protocols. In addition, prospective clinical studies are needed to determine whether cIAP1 is indeed a reliable clinical predictor of outcome for individual patients treated with radiotherapy.

Immunohistochemical studies have been carried out elsewhere to assess the role of cIAP1 as a predictor of prognosis for cervical cancer (19) and of response to chemotherapy for lung cancer (25). However, neither of those studies found any clinical significance of cIAP1 as a molecular predictor for those diseases. One explanation for the conflict between those results and ours is that we focused only on cases treated with uniform radiotherapy protocols. A predominance of overexpressed IAPs may indeed protect tumors from radiotherapy-induced cell death and contribute to the radioresistance of individual cancers, and analyses using other types of tumors treated with uniform radiotherapy procedures may be useful to determine the likelihood of this possibility. Another explanation might be that nuclear and cytoplasmic cIAP1 immunoreactivities were evaluated differently from one study to another. Although cIAP1 immunoreactivity has been detected in both cytoplasm and nuclei of tumor cells (19, 25, 34), the differences between the two staining patterns are not always assessed.

Table 2 Cox proportional hazard regression analysis for local recurrence-free survival and overall survival

<table>
<thead>
<tr>
<th></th>
<th>Local recurrence-free survival</th>
<th>Overall survival</th>
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<tbody>
<tr>
<td></td>
<td>Univariate</td>
<td>Multivariate</td>
</tr>
<tr>
<td></td>
<td>RR (95% CI)</td>
<td>P</td>
</tr>
<tr>
<td>Stage</td>
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<tr>
<td>1–III</td>
<td>1.00 (0.99–1.01)</td>
<td>0.36</td>
</tr>
<tr>
<td>IV</td>
<td>3.26 (1.34–7.91)</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Age (yrs)</td>
<td></td>
<td></td>
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<tr>
<td>≥55</td>
<td>1.00 (0.0055)</td>
<td>0.0026</td>
</tr>
<tr>
<td>&lt;55</td>
<td>3.27 (1.41–7.58)</td>
<td>&lt;0.0001</td>
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<td>Nuclear cIAP1 staining</td>
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<tr>
<td>0 or 1+</td>
<td>1.00 (0.0018)</td>
<td>0.0026</td>
</tr>
<tr>
<td>2+</td>
<td>3.65 (1.62–8.22)</td>
<td>0.0018</td>
</tr>
<tr>
<td></td>
<td>2.04 (0.91–4.58)</td>
<td>0.082</td>
</tr>
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* RR, relative risk/hazard ratio; CI, confidence interval.
* P values are from two-sided tests and were statistically significant when <0.05. Statistically significant values are in **boldface type**.
* Scores of cIAP1 expression were classified as 0, 1+, and 2+, as described in “Materials and Methods.”
Nuclear localization has been observed for two other members of the IAP family, XIAP (19) and survivin (35). The importance of differences between nuclear and cytoplasmic survivin immunoreactivities as predictors of prognosis has been reported in gastric cancers, where nuclear positivity correlated with a favorable prognosis but cytoplasmic positivity did not correlate with any factors associated with either progression or prognosis (35). Others have reported nuclear localization of caspases 3 and 9, targets for the antiapoptotic cIAP1 (36–41), suggesting that binding or interaction between cIAP1 and the caspases may occur in the nucleus and be associated with its antiapoptotic activity. Nuclear protein XAF1 (X-linked inhibitor of apoptosis-associated factor-1), which directly binds to XIAP1 and results in XIAP sequestration in nuclear inclusions, was identified quite recently (42). On the other hand, cIAP1 may also interact with uncharacterized molecule(s) other than caspases to distribute cIAP1 from the cytoplasm to the nucleus and regulate its antiapoptotic function. Published data and our findings reported here indicate a need for additional investigation of the molecular mechanism of cIAP1 in tumorigenesis and of its role in resistance to apoptotic stimuli.

To summarize, we have demonstrated that cIAP1 is overexpressed via an amplification mechanism in cell lines as well as primary CSCCs, although relatively infrequently, and that cell lines overexpressing cIAP1 are resistant to radiation-induced cell death. In addition, elevated expression of cIAP1 in the nucleus showed good correlation with unfavorable prognosis after radiotherapy in patients with CSCCs. We proved statistically that nuclear cIAP1 staining is an independent predictive factor, especially for local recurrence after radiotherapy. These findings indicate that cIAP1 may play an important role in the development and progression of CSCCs, although the molecular mechanism needs to be investigated further. In the meantime, cIAP1 may be a novel target for treatment of this disease and could serve as a prognostic marker for individual outcomes after radiotherapy.

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