

Constitutive Activation of Caspase-3 in Non-Apoptotic Oral Squamous Cell Carcinoma Cells

Wataru Heshiki¹, Kei Tomihara^{1*}, Manabu Yamazaki², Naoya Arai³, Kenji Nakamori¹ and Makoto Noguchi¹

¹Department of Oral and Maxillofacial Surgery, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama 2630 Sugitani, Toyama City, Japan

²Division of Oral Pathology, Department of Tissue Regeneration and Reconstruction, Niigata University, Graduate School of Medical and Dental Sciences, Niigata City, Japan

³Department of Oral and Maxillofacial Surgery, Division of Reparative and Regenerative Medicine, Institute of Medical Science, Mie University Graduate School of Medicine, Tsu, Mie, Japan

Abstract

Background: Although caspase-3 is a key molecule for apoptosis induction, recent evidence has suggested its protumoral role in various human malignancies. The aim of the present study was to investigate the expression of cleaved caspase-3 (the active form of caspase-3) in both clinical samples and cell lines from oral squamous cell carcinomas (OSCCs) and elaborate on its contribution to the protumor role in oral cancer.

Methods: The expression of cleaved caspase-3 was immunohistochemically evaluated in samples from 30 patients with OSCCs. The samples were either from biopsies or surgically-resected specimens with a mix of clinical stages and tumor site origins. The expression of cleaved caspase-3 was further examined in three OSCC cell lines.

Results: In addition to apoptotic cancer cells, all the cases of OSCCs demonstrated a surprisingly positive expression of cleaved caspase-3. A diffuse, cytoplasmic pattern was particularly prominent in *in situ* carcinoma cells, invasive carcinoma cells, and metastatic cancer cells that lacked apoptotic morphology. On the other hand, non-neoplastic, normal epithelial cells were completely negative for cleaved caspase-3. In all the OSCC cell lines studied, cleaved caspase-3 was expressed in the cytoplasm and nucleus of cancer cells. Flow cytometric analysis also confirmed that the activation level of caspase-3 in non-apoptotic cancer cells was relatively lower than that in apoptotic cancer cells. Moreover, caspase-3 inhibition by caspase-3 specific inhibitor decreased the proliferation of OSCC cells.

Conclusions: Because cleaved caspase-3 is selectively expressed in non-apoptotic OSCC cells and is associated with cell proliferation, these findings implicate caspase-3 signaling in promoting the progression of oral cancer.

Keywords: Caspase-3 activation; Non-apoptotic role; Oral cancer; Immunohistochemical analysis; Fluorogenic caspase-3 substrate

Introduction

Caspase-3 is a member of the cysteine protease family and plays a critical role in the regulation of programmed cell death (apoptosis) [1]. Caspase-3 expression has been extensively studied in many cancers. The positive correlation between its expression and a favorable prognosis has been reported in several cancers, suggesting its use as a prognostic marker for cancers [2]. In oral squamous cell carcinomas (OSCCs), increased caspase-3 expression has been shown to inversely correlate with cell differentiation [3].

Cleaved caspase-3, the active form of caspase-3, is well known as a marker for cells undergoing apoptosis in both normal tissue and cancer cells [1]. Given this, it is now widely accepted that cleaved caspase-3 staining is useful in scoring the apoptotic index. However, it has also been shown that caspase-3 signaling contributes to non-apoptotic processes such as the differentiation of various cell types, including lens cells, monocytes, muscle cells, osteoblasts, neural stem cells, glial cells, and embryonic stem cells [4-10]. Moreover, there have been a number of notable studies demonstrating its non-apoptotic role in cancer, which has been linked to a protumor activity such as tumor cell proliferation, cell migration, and tumor repopulation after cytotoxic therapy [11-16]. However, only a limited number of studies have been published regarding the non-apoptotic, protumoral role of caspase-3. It is therefore necessary that additional studies in other cancer cell

types are conducted to support this paradoxical role of caspase-3 in cancer biology.

No studies have yet addressed the non-apoptotic role of this molecule in OSCCs. In the present study, we have addressed whether caspase-3 has a non-apoptotic role in facilitating tumor promotion in OSCCs. In all the tumors studied, immunohistochemical analysis revealed that cleaved caspase-3 was selectively expressed in carcinoma cells without apoptotic morphology, but not in adjacent, normal epithelial cells. Furthermore, in all OSCC cell lines studied, it was confirmed that cleaved caspase-3 was expressed in the cytoplasm and nucleus of cancer cells and that the activation level of caspase-3 in non-apoptotic cancer cells was relatively lower than that in apoptotic cancer

***Corresponding author:** Kei Tomihara, DDS, PhD, Department of Oral and Maxillofacial Surgery, Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, 2630 Sugitani, Toyama city, Toyama 930-0194, Japan, Tel: +81-76-434-7383 (ex 7383); E-mail: tomihara@med.u-toyama.ac.jp

Received December 03, 2014; **Accepted** February 12, 2015; **Published** February 28, 2015

Citation: Heshiki W, Tomihara K, Yamazaki M, Arai N, Nakamori K, et al. (2015) Constitutive Activation of Caspase-3 in Non-Apoptotic Oral Squamous Cell Carcinoma Cells. J Cancer Sci Ther 7: 075-080. doi:10.4172/1948-5956.1000328

Copyright: © 2015 Heshiki W, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

cells. Thus, caspase-3 signaling may be involved in a non-apoptotic mechanism in oral cancer.

Materials and Methods

Clinical samples

This study included 30 patients who received surgical treatment for OSCC at the Department of Oral and Maxillofacial Surgery (Graduate School of Medicine and Pharmaceutical Sciences for Research, University of Toyama, Toyama, Japan) between April 2007 and March 2014. An informed consent statement approved by our institutional review board was obtained from all the patients prior to any examination or treatment. The sites of primary carcinoma were the tongue ($n=10$), maxillary gingiva ($n=6$), mandibular gingiva ($n=5$), floor of the mouth ($n=3$), and buccal mucosa ($n=6$). In total, 5 patients were diagnosed with stage I primary OSCC, 9 with stage II, 9 with stage III, and 7 with stage IV.

Their tissue specimens taken as biopsy or surgical resection were routinely fixed in 10% formalin and embedded in paraffin. After histopathological review, one representative block containing non-neoplastic epithelia, epithelial dysplasia, carcinoma *in situ* (CIS), and invasive carcinoma was selected in each case. In addition, specimens of cervical lymph nodes with metastatic carcinoma were also included.

Immunohistochemical analysis

Sections of 3 μ m thickness from paraffin-embedded tumor samples were investigated using immunohistochemical analysis. For antigen retrieval, the sections were immersed in 0.01 M sodium citrate buffer (pH 6.0) and heated for 10 min in a microwave. After allowing the sections to cool to room temperature, they were then incubated with 3% hydrogen peroxide for 30 min to block endogenous peroxidase activity. The sections were subsequently incubated overnight with rabbit polyclonal antibodies against cleaved caspase-3 (1:300; Cell Signaling Technology, Beverly, MA, USA) at 4°C. After incubation with the primary antibodies, the sections were incubated with biotinylated secondary antibodies for 15 min followed by a streptavidin-biotin-peroxidase complex (Nichirei Biosciences, Tokyo, Japan) for 30 min at room temperature. Reactions were catalyzed with a 3,3'-diaminobenzidine substrate solution (Dako, Glostrup, Denmark), and the sections were counterstained with hematoxylin. As a control, the sections were stained with either normal rabbit serum or without exposure to a primary antibody.

Cell lines and reagents

The human oral squamous cell carcinoma (OSCC) cell lines HSC-3, HSC-4, and SAS were purchased from the Cell Bank of Human Science Resources of Japan (CBHSRJ). The human keratinocyte cell line HaCat was kindly provided by Dr. Shimizu, Department of Dermatology, University of Toyama. The cells were maintained in complete Dulbecco's modified Eagle's medium. Docetaxel was purchased from Toronto Research Chemicals (North York, ON, Canada), and the caspase-3 inhibitor z-DEVD-fmk was purchased from R&D systems (Minneapolis, MN, USA).

Assessment of cell apoptosis and caspase-3 activity by flow cytometry

Apoptotic cells were detected using Annexin V (BD PharMingen, San Diego, CA, USA), according to the manufacturer's instructions. The cells were harvested and stained using a fluorescein isothiocyanate-labeled anti-Annexin antibody. The samples were then stained

with propidium iodide and analyzed with a FACS Canto II (Becton Dickinson, San Jose, CA, USA). Apoptotic cells were calculated based on the percentage of Annexin V-positive cells. Annexin-positive/PI-negative cells were considered as early apoptotic cells, whereas Annexin V-positive/PI-positive cells were considered as late apoptotic cells.

To assess caspase-3 activity, the cells were fixed with 4% paraformaldehyde/PBS (Wako Pure Chemical Industries, Osaka, Japan) for 10 min at 37°C, pre-chilled on ice for 1 min, and then permeabilized by slowly adding ice-cold methanol (Nakarai Tech, Tokyo, Japan) to a final concentration of 90%. Thirty minutes later, the cells were washed and stained with an anti-cleaved caspase-3 antibody (Alexa Fluor[®] 488 conjugated anti-cleaved caspase-3, Cell Signaling Technology) or isotype control (Alexa Fluor[®] 488 conjugated isotype control, Cell Signaling Technology) at room temperature for 1 h. The samples were then analyzed by a FACS Canto II, and the mean fluorescence intensity of the cleaved caspase-3 signal was measured using FLOWJO (FLOWJO, LCC, Ashland, OR, USA) software.

Assessment of cell proliferation

Cells were seeded into 96-well plates at 1.5×10^4 cells per well in the absence or presence of 200 μ mol/L caspase-3 inhibitor z-DEVD-fmk, and the viability of adherent cells was measured at 24, 48, 72, and 96 hours after seeding in the wells. To measure the viability of adherent cells, tetrazolium salt WST-1 (4-[3-(4-iodophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolol]-1, 3-benzene disulfonate) premix (Takara Bio, Otsu, Shiga, Japan) was added to each well. The cleavage of WST-1 into formazan by metabolically active cells was measured by scanning the plates at 450 nm in a microtiter plate reader.

Statistical analysis

Group comparisons were made using a Student's t-test. A p -value of <0.05 was considered significant.

Results

Cleaved caspase-3 was expressed in all oral squamous carcinoma (OSCC) cases analyzed

We examined the immunohistochemical localization of cleaved caspase-3. To confirm the specificity of the antibodies against cleaved caspase-3, we used lymph node specimens as a positive control. As a result, positive staining was obtained in apoptotic lymphocytes in the germinal centers, but not in other immune cells lacking apoptotic features (Figure 1A), indicating the specificity of the antibodies. In non-neoplastic squamous epithelia adjacent to carcinoma cells, positive staining for cleaved caspase-3 was not detected in all cases (Figure 1B). In the epithelial dysplasia, however, cleaved caspase-3 was localized to the lower half of the epithelia layer (Figure 1C), corresponding to the proliferating area of the parabasal cells. In CIS, immunoreactivity for cleaved caspase-3 was observed in almost the entire layer except for the superficial keratinized cells (Figure 1D). In invasive carcinoma, positive staining was extensively observed in carcinoma cells in all cases (Figure 1E). The cytoplasm of carcinoma cells lacking apoptotic figures was diffusely positive for cleaved caspase-3. In addition, apoptotic carcinoma cells stained more strongly for cleaved caspase-3 than viable carcinoma cells (Figure 1F). Moreover, some heterogeneity was present in the staining intensity, and some areas, including those containing less keratinized carcinoma cells, showed less intense immunoreaction (Figure 1G). Metastatic carcinoma cells of the cervical lymph nodes were also positive for cleaved caspase-3 (Figure 1H). However, there was no apparent correlation between cleaved caspase-3 expression and

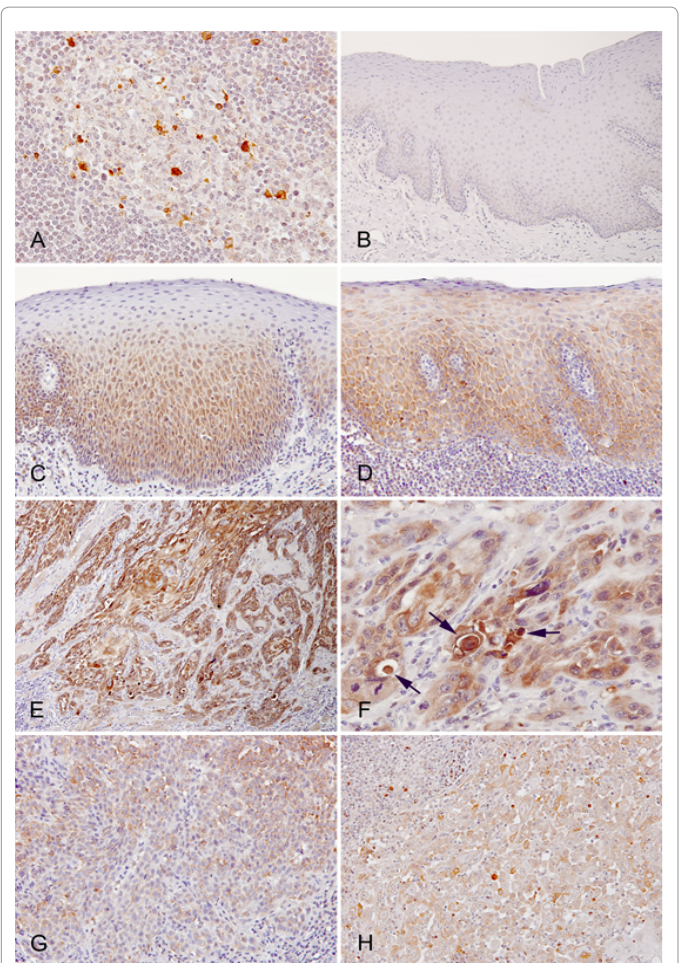


Figure 1: Immunohistochemical localization of cleaved caspase-3 in non-neoplastic epithelia, epithelial dysplasia, carcinoma *in situ* (CIS), invasive squamous cell carcinoma (SCC), and metastatic SCC in samples of lymph nodes. The representative staining pattern of cleaved caspase-3 in the germinal center of non-metastatic lymph node (A), non-neoplastic epithelium (B), epithelial dysplasia (C), CIS (D), invasive carcinoma (E-G), metastatic carcinoma samples (H); (A-H) immunoperoxidase stain for cleaved caspase-3. Specific positive staining for cleaved caspase-3 was confirmed in apoptotic lymphocytes in the germinal centers of lymph nodes (A); however, no obvious positive staining in non-neoplastic squamous epithelia was observed (B). In epithelial dysplasia, cleaved caspase-3 was localized in the proliferative foci of parabasal cells in the lower half of the epithelia (C). In CIS, positive staining was observed in almost the entire layer of the epithelia except for the superficial keratinized cells (D). In invasive carcinoma, extensive positive staining was observed in carcinoma cells (E). The cytoplasm of carcinoma cells lacking apoptotic figures was diffusely positive for cleaved caspase-3 in addition to apoptotic carcinoma cells showing a rounding of their cytoplasm and nuclear condensation (F). Arrows indicate apoptotic carcinoma cells. Staining intensities varied from case to case, and in particular, less keratinized carcinoma cells displayed weak staining (G). Metastatic carcinoma cells in the lymph nodes were also positive for cleaved caspase-3 (H).

any clinicopathological characteristics including tumor stage, tumor location, or the differentiation status of the tumor (Table 1).

Cleaved caspase-3 was expressed by OSCC cell lines and was associated with cell proliferation

In order to better understand the specific localization of cleaved caspase-3 in carcinoma cells, the intracellular distribution of the molecule was examined under a fluorescent microscope in three OSCC cell lines HSC-3, HSC-4, and SAS, and nontumorigenic keratinocyte

line HaCat cells using a fluorogenic caspase-3 substrate. In all the OSCC cell lines analyzed, cleaved caspase-3 was distributed in both the cytoplasm and nuclei (Figure 2). In contrast, no distribution of the molecule was observed in HaCat cells (Figure 2). Furthermore, we also evaluated the activation level of caspase-3 in these cell lines by staining for intracellularly-cleaved caspase-3 expression using flow cytometry. In the entire cancer cell lines analyzed, and despite the fact that levels of apoptosis were extremely low (Figure 3A), cleaved caspase-3 expression was observed in the cancer cells; this activation was significantly impaired by treatment with a caspase-3 inhibitor (Figure 3B). On the other hand, as a positive control for induced-

| characteristics | | n |
|-----------------|---------------------------|---------|
| median age | | 68.5 |
| (range) | | (46-93) |
| sex | male | 15 |
| | female | 15 |
| tumor location | tongue | 10 |
| | mandibular gingiva | 6 |
| | maxillary gingiva | 5 |
| | floor of the mouse | 3 |
| | buccal mucosa | 6 |
| differentiation | well-differentiated | 18 |
| | moderately-differentiated | 10 |
| | poorly-differentiated | 2 |
| clinical stage | I | 5 |
| | II | 9 |
| | III | 9 |
| | IV | 7 |

Table 1: Characteristics of patients with oral squamous cell carcinoma.

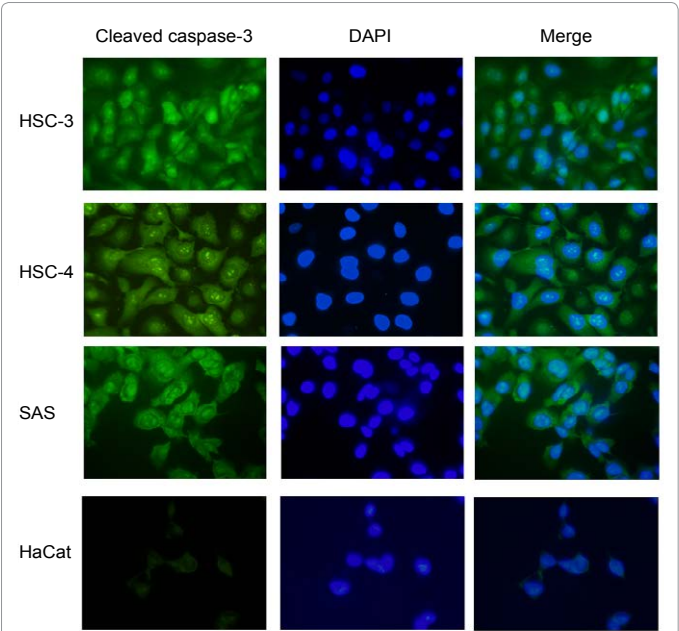


Figure 2: Subcellular localization of cleaved, active caspase-3 in oral squamous cell carcinoma (OSCC) cells. HSC-3, HSC-4, SAS, and HaCat cells were cultured on glass chamber slides overnight at 37°C. They were then analyzed using CellEvent® Caspase-3/7, a green detection cell-permeable reagent for the intracellular distribution of cleaved caspase-3, under a fluorescence microscope. Green indicates cleaved caspase-3 and blue indicates nuclei.

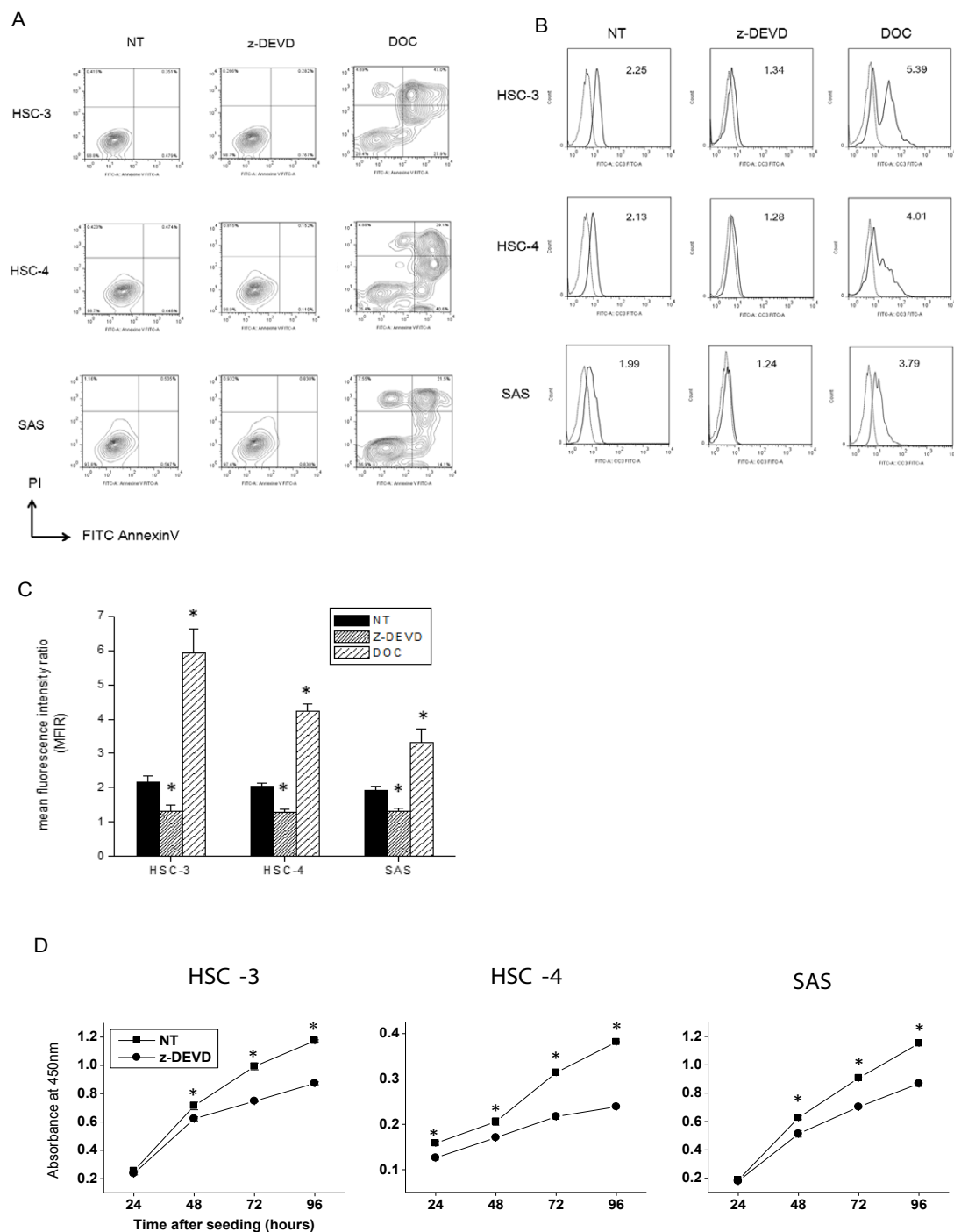


Figure 3: Cleavage activity of caspase-3 was observed in oral squamous cell carcinoma (OSCC) cells without apoptosis. (A) Analysis of apoptotic cell death in OSCC cell lines. OSCC cells were grown in culture medium with 10% fetal bovine serum (FBS). Control cells for caspase-3 inhibition were treated with 200 $\mu\text{mol/L}$ caspase-3/7 inhibitor z-DEVD-fmk for 12 h. The control cells for apoptotic cell death were treated with 50 μM docetaxel for 12 h. The cells were then examined by staining with both Annexin V and propidium iodide (PI). The X-axis represents the Annexin-related fluorescence and the Y-axis represents the PI-related fluorescence. The cells in the lower right and upper right represent early apoptotic cells and late apoptotic cells, respectively. (B) Analysis of active caspase-3 in OSCC cell lines by flow cytometry. The OSCC cells were grown in culture medium with 10% FBS. The control cells for caspase-3 inhibition were treated with z-DEVD-fmk for 12 h. The control cells for apoptotic cell death were treated with 50 μM docetaxel for 12 h. The cells were then examined by intracellular staining with either isotype control antibody or anti-cleaved caspase-3 antibody. Experiments were performed in triplicate, and representative data from one experiment is shown. The dashed lines represent staining with the isotype control antibody. Solid lines represent staining with the anti-cleaved caspase-3. The number in each panel represents the mean fluorescence intensity ratio (MFIR). (C) The mean \pm SD of the fluorescent intensity of the cleaved caspase-3 was analyzed. An asterisk indicates a significant difference in the MFIR compared with the non-treated cells in each cell line. ($p < 0.05$); NT, nontreated; DOC, docetaxel. (D) Growth inhibitory effect of caspase-3 inhibition on OSCC cells. The OSCC cells were incubated in the absence or presence of z-DEVD-fmk, and the time course of proliferation of adherent OSCC cells was measured by the WST-1 cell proliferation assay system. The mean \pm SD from sextuplicate determinations are shown. An asterisk indicates a significant difference between two groups. ($p < 0.05$)

caspase-3 activation, the cells treated with the cytotoxic agent docetaxel demonstrated significantly higher levels of caspase-3 activity when undergoing apoptosis compared with those in non-treated cancer cells (Figure 3A and B).

To directly determine the effect of caspase-3 activity on OSCC cells, we also evaluated the proliferation of OSCC cells in the presence or absence of caspase-3 inhibitor. As expected, caspase-3 inhibition significantly decreased proliferation of OSCC cells (Figure 3D). Overall, these data provide strong evidence supporting the hypothesis that caspase-3 signaling facilitates oral cancer progression.

Discussion

In the present study, we examined the activation of caspase-3 in oral cancer using both OSCC clinical samples and cell lines. In all the OSCC cases studied, the expression of cleaved caspase-3 was observed in non-apoptotic OSCC cells in addition to apoptotic cancer cells. Moreover, cleaved caspase-3 was also expressed in the OSCC cell lines in the absence of apoptotic cell death. Although it has long been recognized that caspase-3 plays an important role in apoptosis induction, recent evidence has suggested that this molecule also participates in a variety of non-apoptotic biological processes [4-16]. Several studies have suggested that caspase-3 is involved in tumor aggressiveness by promoting cell motility, invasiveness, and proliferation of cancer cells. For example, Gdynia et al. reported that the constitutive, low level expression of activated caspase-3 contributes to the aggressiveness of brain tumors [13]. They also revealed, using immunohistochemistry, that human glioblastomas exhibit a weak to moderate activity of caspase-3 in glioblastoma cells without any apoptotic morphology. Moreover, *in vitro* studies revealed that inhibition of caspase-3 activity by the use of specific inhibitors or siRNA decreases the rate of migration and invasiveness of glioblastoma cells. In agreement with this, the protumoral role of active caspase-3 has also been demonstrated in melanomas [15]. Liu et al. revealed that basal caspase-3 activity is detected in melanoma cells, whereas the inhibition of caspase-3 activity also decreases the rate of migration, invasion, and vasculogenic mimicry in these cells *in vitro*. In an additional study by the same authors, it was shown that caspase-3 signaling stimulates tumor repopulation during cytotoxic therapy [14]. In their *in vitro* study, they revealed that radiation-induced caspase-3 activation stimulates either cancer cells or stromal cells to release prostaglandin E2, thus promoting tumor cell repopulation. In consideration of these results, it seems that caspase-3 activation has an important role in non-apoptotic, protumoral processes.

Apoptosis is well known as a process of programmed cell death, which plays an important role in the development of multicellular organisms and in the maintenance of cellular homeostasis. When a cell is either damaged or no longer required, it can be eliminated from the body through the mechanism of apoptosis. Thus, during tumor progression, apoptosis operates as an important biological phenomenon in eliminating cancer cells. Apoptotic cell death is frequently observed in various cancers, linking the biological property of tumors with clinical outcomes. In certain cancers, high apoptotic index has been shown to correlate with worse clinical outcomes and a higher grade malignancy based on a high mitotic index, high-grade histology, and high Ki-67 labeling index [17-20]. Taken together, these results suggest that increased levels of apoptosis are associated with tumor progression with a high turnover of tumor cells. On the other hand, a general consensus is that apoptotic signaling is repressed in cancer cells through the inhibition of multiple factors downstream of these signaling cascades. Several studies have revealed that cancer cells

abundantly express anti-apoptotic molecules whereas the expression of pro-apoptotic molecules is often impaired [21-26]. Moreover, the expression of various molecules involved in regulating apoptosis, such as Bcl-XL, survivin, a death receptor such as Fas or TRAIL receptors, is modulated by alternative splicing [27-30]. Given this, it seems likely that apoptosis is controlled through a complex mechanism during cancer progression. Previous studies have revealed that the extent of caspase-3 activation appears to be a balance between the protumoral function of the molecule and its ability to induce apoptosis [11,12]. Moreover, as suggested in earlier studies, basal caspase-3 activation does not induce apoptosis but rather promotes tumorigenesis [13,15].

Consistent with these findings, the results of our study have revealed that caspase-3 activation in non-apoptotic OSCC cells was relatively lower than that in apoptotic cancer cells. Thus, it may be possible that a low level of activation is insufficient to induce the apoptosis of cancer cells but high enough to facilitate the proliferation of cancer cells.

The most critical question is why caspase-3 is constitutively activated in cancer cells. It is well recognized that cancer cells receive numerous stimulations to activate apoptotic signaling via various cellular stresses or death receptors such as Fas and TRAIL receptors. However, while it has been shown that Fas elicits apoptotic signals and functions in various pathological processes, recent evidence suggests Fas facilitates tumor growth and was found to be associated with extremely poor outcomes for patients in several cancer types [31-34]. Therefore, it may be possible that Fas is also related to the non-apoptotic, basal caspase-3 activity in cancer cells. We consistently observed that anti-Fas antibody treatment stimulated the proliferation of OSCC cells in a dose-dependent manner, although it remains unclear if Fas signaling is directly associated with a constitutive, low level of caspase-3 activation (Heshiki, et al. unpublished data).

In conclusion, we demonstrated non-apoptotic caspase-3 activation in both OSCC clinical samples and OSCC cell lines, although the level of activation was relatively lower than that of apoptotic cancer cells.

In oral cancer, this basal caspase-3 signaling may be utilized for the proliferation of cancer cells but not apoptosis induction. The concept of compensatory proliferation, wherein apoptotic cells stimulate neighboring cells to proliferate, has been demonstrated in a number of cell types, including cancer cells [35,36]. Taken together, a low level of caspase-3 activation, which although insufficient to induce the genuine apoptosis of cancer cells, may be utilized for compensatory proliferation and facilitate the promotion of tumor progression in oral cancer. However, further investigations would be necessary to elucidate the detailed mechanism of how a low level of activated caspase-3 facilitates tumor progression in oral cancer. In the present study, we showed that oral cancer cells can be effectively recognized by the selective expression of cleaved caspase-3, a finding, at least in part, which may have clinical and therapeutic implications in oral cancer.

Acknowledgments

We thank Dr. Oura Takehiro, Dr. Inoue Sayaka, and Dr. Noto Zenko for their technical assistance. This work was supported by Japan Society for the Promotion of Science (JSPS) KAKENHI Grant number 25861922.

The authors made no disclosures.

References

1. Budihardjo I, Oliver H, Lutter M, Luo X, Wang X (1999) Biochemical pathways of caspase activation during apoptosis. *Annu Rev Cell Dev Biol* 15: 269-290.

2. Fiandalo MV, Kyprianou N (2012) Caspase control: protagonists of cancer cell apoptosis. *Exp Oncol* 34: 165-175.
3. Hague A, Eveson JW, MacFarlane M, Huntley S, Janghra N, et al. (2004) Caspase-3 expression is reduced, in the absence of cleavage, in terminally differentiated normal oral epithelium but is increased in oral squamous cell carcinomas and correlates with tumour stage. *J Pathol* 204: 175-182.
4. Ishizaki Y, Jacobson MD, Raff MC (1998) A role for caspases in lens fiber differentiation. *J Cell Biol* 140: 153-158.
5. Sordet O, Rébé C, Plenchette S, Zermati Y, Hermine O, et al. (2002) Specific involvement of caspases in the differentiation of monocytes into macrophages. *Blood* 100: 4446-4453.
6. Fernando P, Kelly JF, Balazsi K, Slack RS, Megeney LA (2002) Caspase 3 activity is required for skeletal muscle differentiation. *Proc Natl Acad Sci U S A* 99: 11025-11030.
7. Miura M, Chen XD, Allen MR, Bi Y, Gronthos S, et al. (2004) A crucial role of caspase-3 in osteogenic differentiation of bone marrow stromal stem cells. *J Clin Invest* 114: 1704-1713.
8. Fernando P, Brunette S, Megeney LA (2005) Neural stem cell differentiation is dependent upon endogenous caspase 3 activity. *FASEB J* 19: 1671-1673.
9. Oomman S, Strahlendorf H, Dertien J, Strahlendorf J (2006) Bergmann glia utilize active caspase-3 for differentiation. *Brain Res* 1078: 19-34.
10. Fujita J, Crane AM, Souza MK, Dejosez M, Kyba M, et al. (2008) Caspase activity mediates the differentiation of embryonic stem cells. *Cell Stem Cell* 2: 595-601.
11. Mukai M, Kusama T, Hamanaka Y, et al. (2005) Cross talk between apoptosis and invasion signaling in cancer cells through caspase-3 activation. *Cancer Res* 65: 9121-9125.
12. Zhao X, Wang D, Zhao Z, Xiao Y, Sengupta S, et al. (2006) Caspase-3-dependent activation of calcium-independent phospholipase A2 enhances cell migration in non-apoptotic ovarian cancer cells. *J Biol Chem* 281: 29357-29368.
13. Gdynia G, Grund K, Eckert A, Böck BC, Funke B, et al. (2007) Basal caspase activity promotes migration and invasiveness in glioblastoma cells. *Mol Cancer Res* 5: 1232-1240.
14. Huang Q, Li F, Liu X, Li W, Shi W, et al. (2011) Caspase 3-mediated stimulation of tumor cell repopulation during cancer radiotherapy. *Nat Med* 17: 860-866.
15. Liu YR, Sun B, Zhao XL, Gu Q, Liu ZY, et al. (2013) Basal caspase-3 activity promotes migration, invasion, and vasculogenic mimicry formation of melanoma cells. *Melanoma Res* 23: 243-253.
16. Donato AL, Huang Q, Liu X, Li F, Zimmerman MA, et al. (2014) Caspase 3 promotes surviving melanoma tumor cell growth after cytotoxic therapy. *J Invest Dermatol* 134: 1686-1692.
17. Langlois NE, Eremin O, Heys SD (2000) Apoptosis and prognosis in cancer: rationale and relevance. *J R Coll Surg Edinb* 45: 211-219.
18. Lipponen PK, Aaltomaa S (1994) Apoptosis in bladder cancer as related to standard prognostic factors and prognosis. *J Pathol* 173: 333-339.
19. Lipponen P1 (1999) Apoptosis in breast cancer: relationship with other pathological parameters. *Endocr Relat Cancer* 6: 13-16.
20. Krajewski S, Krajewska M, Turner BC, Pratt C, Howard B, et al. (1999) Prognostic significance of apoptosis regulators in breast cancer. *Endocr Relat Cancer* 6: 29-40.
21. Tomita M, Matsuzaki Y, Edagawa M, Shimizu T, Hara M, et al. (2003) Prognostic significance of bcl-2 expression in resected pN2 non-small cell lung cancer. *Eur J Surg Oncol* 29: 654-657.
22. Callagy GM, Pharoah PD, Pinder SE, Hsu FD, Nielsen TO, et al. (2006) Bcl-2 is a prognostic marker in breast cancer independently of the Nottingham Prognostic Index. *Clin Cancer Res* 12: 2468-2475.
23. Droin N, Guéry L, Benikhlef N, Solary E (2013) Targeting apoptosis proteins in hematological malignancies. *Cancer Lett* 332: 325-334.
24. Lebedeva I, Rando R, Ojwang J, Cossum P, Stein CA (2000) Bcl-xL in prostate cancer cells: effects of overexpression and down-regulation on chemosensitivity. *Cancer Res* 60: 6052-6060.
25. Gobé G, Rubin M, Williams G, Sawczuk I, Buttyan R (2002) Apoptosis and expression of Bcl-, Bcl-XL, and Bax in renal cell carcinomas. *Cancer Invest* 20: 324-332.
26. Cho HJ, Kim JK, Kim KD, Yoon HK, Cho MY, et al. (2006) Upregulation of Bcl-2 is associated with cisplatin-resistance via inhibition of Bax translocation in human bladder cancer cells. *Cancer Lett* 237: 56-66.
27. Schwerk C, Schulze-Osthoff K (2005) Regulation of apoptosis by alternative pre-mRNA splicing. *Mol Cell* 19: 1-13.
28. Li C, Kato M, Shiue L, Shively JE, Ares M, Jr, et al. (2006) Cell type and culture condition-dependent alternative splicing in human breast cancer cells revealed by splicing-sensitive microarrays. *Cancer Res* 66: 1990-1999.
29. David CJ, Manley JL (2010) Alternative pre-mRNA splicing regulation in cancer: pathways and programs unhinged. *Genes Dev* 24: 2343-2364.
30. Krieg A, Mersch S, Wolf N, Stoecklein NH, Verde PE, am Esch JS 2nd, et al. (2013) Expression of TRAIL-splice variants in gastric carcinomas: identification of TRAIL-? as a prognostic marker. *BMC Cancer* 13: 384.
31. Barnhart BC, Legembre P, Pietras E, Bubici C, Franzoso G, et al. (2004) CD95 ligand induces motility and invasiveness of apoptosis-resistant tumor cells. *EMBO J* 23: 3175-3185.
32. Li H, Fan X, Stoicov C, Liu JH, Zubair S, Tsai E, et al. (2009) Human and mouse colon cancer utilizes CD95 signaling for local growth and metastatic spread to liver. *Gastroenterology* 137: 934-44.
33. Zhang Y, Liu Q, Zhang M, Yu Y, Liu X, et al. (2009) Fas signal promotes lung cancer growth by recruiting myeloid-derived suppressor cells via cancer cell-derived PGE2. *J Immunol* 182: 3801-3808.
34. Chen L, Park SM, Tumanov AV, Hau A, Sawada K, et al. (2010) CD95 promotes tumour growth. *Nature* 465: 492-496.
35. Fan Y, Bergmann A (2008) Distinct mechanisms of apoptosis-induced compensatory proliferation in proliferating and differentiating tissues in the *Drosophila* eye. *Dev Cell* 14: 399-410.
36. Maeda S, Kamata H, Luo JL, Leffert H, Karin M (2005) IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* 121: 977-990.