Role of Myeloid Cell Leukemia-1 in Cell Growth of Squamous Cell Carcinoma

Masahide Nagata¹,², Koichiro Wada¹,*, Atsushi Nakajima³, Noriko Nakajima⁴, Morio Kusayama¹,², Tomotake Masuda⁵, Seiji Iida⁵, Masaya Okura⁵, Mikihiko Kogo⁵, and Yoshinori Kamisaki¹

¹Department of Pharmacology and ²The First Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry, Osaka University, 1-8 Yamadaoka, Suita, Osaka 565-0871, Japan
³Division of Gastroenterology, Yokohama City University School of Medicine, 3-9 Fuku-ura, Yokohama 236-0004, Japan
⁴Department of Pathology, National Institute of Infectious Diseases, 1-23-1 Toyama, Shinjuku-ku, Tokyo 162-8640, Japan

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Abstract. Myeloid cell leukemia-1 (Mcl-1), a member of the B-cell lymphoma-2 (Bcl-2) family, has been reported to be a critical survival factor in hematopoietic cells, yet little data exists for a role of Mcl-1 in human oral squamous cell carcinoma (SCC). A high level expression of Mcl-1 was observed in tumor cells of human primary SCC, lymph node metastasis tissues, and SCC cell lines. We manipulated expression of Mcl-1 protein in SCC cells by small interfering RNA (siRNA) for Mcl-1 and observed that Mcl-1 siRNA inhibited the growth of SCCs accompanied with apoptosis. Combination therapy of Mcl-1 siRNA and anti-tumor drug drastically inhibited the cell growth in comparison to that in each single treatment. In addition, phosphorylation of focal adhesion kinase (FAK) was decreased by treatment with Mcl-1 siRNA, resulting in decreases in phosphorylation of MEK1/2 and MAPK. The cell growth inhibition caused by knockdown of Mcl-1 was suggested to be mainly a result of suppression of proliferation via the inhibition of intracellular FAK/MAPK signaling pathways. These results imply a potentially important and novel role of the inhibition of Mcl-1 function by the use of specific siRNA in the treatment of SCC.

Keywords: myeloid cell leukemia-1 (Mcl-1), squamous cell carcinoma, apoptosis, small interfering RNA (siRNA), human

Introduction

Human oral squamous cell carcinoma (SCC) is the most common neoplasm in oral cavity cancer and the incidence has recently been increasing (1, 2). The optimal treatment or therapy for early carcinoma of the tongue remains a controversial issue, and surgical operation is still effective therapy for SCC in the field of maxillofacial surgery. However, the recovery of lost function after a large surgical excision is very difficult, and often patients complained of impediment of speech or swallowing (3). Local-regional control of head and neck cancer has improved in recent decades. Nevertheless, overall survival remains largely unchanged (4, 5).

The major reason for this discrepancy is distant metastasis and second neoplasms (6). The incidence of clinically detected distant metastasis of the head and neck ranges from 11% to 23%, and most of the metastasis are observed in the lymph nodes and lungs (7).

Myeloid cell leukaemia-1 (Mcl-1), a member of the B-cell lymphoma-2 (Bcl-2) family, has been found to be a critical survival factor in hematopoietic cells such as human neutrophils, leukemic large granular lymphocytes, and multiple myeloma cell lines (8 – 11). Mcl-1 is considered to block cytochrome c release from mitochondria by interacting with members of the pro-apoptotic Bcl-2 family such as Bim, Bak, and NOXA, resulting in anti-apoptosis. However, the direct role of Mcl-1 on cell proliferation is still unclear.

Recent studies have shown that Mcl-1 is overexpressed in human non-small cell lung cancer cells and hepatocellular carcinoma cells. Namely, upregu-
lated expression of Mcl-1 was observed in 4 out of 9 cases of non-small cell lung cancers, and most of the non-small cell lung cancer cell lines expressed Mcl-1. In addition, it was reported that suppression of Mcl-1 by the treatment with Mcl-1 small interfering RNA (siRNA) increased the sensitivity towards apoptosis induction in two carcinoma cell lines (12, 13).

In this study, we have examined whether Mcl-1 is expressed in primary oral SCC and lymph node metastasis tissues and whether Mcl-1 plays a critical role in tumor cell growth. We, therefore, studied the effects of Mcl-1 siRNA upon the viability of oral SCC cell lines and the usefulness of combination therapy of Mcl-1 siRNA and ordinal anti-tumor drugs.

Materials and Methods

Chemicals and antibodies

Anti-Mcl-1 polyclonal antibody was from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Antibodies against Bcl-2, caspase-3, cleaved-caspase-3, FAK, phosphorylated FAK, phosphorylated MEK1/2, p44/42MAPK, and phosphorylated p44/42MAPK are from Cell Signaling Technologies (Beverly, MA, USA). Cisplatin and 5-fluorouracil (5-FU) were from Wako Pure Chemical Industries, Ltd. (Osaka). Other drugs were reagents grade.

Patients and tissue samples

The study protocol conformed to the ethical guideline of the 1975 Declaration of Helsinki and was approved by the Committee of the Department of Oral and Maxillofacial Surgery, Graduate School of Dentistry, Osaka University. After informed consent, samples of squamous cell carcinoma (SCC) located in the tongue were obtained from surgical resection tissue specimens at the First Department of Oral and Maxillofacial Surgery, Osaka University Dental Hospital between January, 1986 and July, 2008. During this period, 213 of 248 patients were given surgery for tongue carcinoma. Among them, the patients who did not receive preoperative therapy including chemotherapy and radiotherapy were selected. Excluding the non-identified histological differentiation of diagnosis, 30 samples were randomly selected with ten of each histological group as “well-differentiated”, “moderately differentiated”, and “poorly differentiated” (Table 1). In general, the number of patients who were diagnosed with poorly differentiated tumors is much less than those with well-differentiated or moderately differentiated tumors (17 of 248 cases, 6.9%). Within 30 cases, 16 patients were males and 14 were females, and the age range was 30 – 86 years (60.7 ± 2.6 years).

Immunohistochemical staining of Mcl-1

Five-μm sections of paraffin-embedded tissues were mounted on glass slides, and the expression of Mcl-1 within tissues was detected by standard immunohistochemical techniques using anti-Mcl-1 specific polyclonal antibody. The Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) was used with a 3,3’-diaminobenzidine substrate kit, according to the manufacturer’s instructions. The staining endpoint was determined when the standard tissue sections were constantly stained with the intensity level of +++, as described previously (14).

The intensity of the immunohistochemical staining with anti-Mcl-1 antibody was evaluated by scoring the staining reaction into four groups: weak (−), moderate (+), strong (++), and very strong (+++) cytoplasmic staining intensity (14). To check the reproducibility of the evaluation system for the immunohistochemical staining of the Mcl-1 proteins, another oral surgeon and pathologist who were unaware of the original assessment re-evaluated the results of staining according to the system above. Tumor areas were confirmed by both the pathologist and surgeon under a microscope. Non-tumor areas were selected as comparatively normal areas that were well-separated and far away from the tumor areas and confirmed by the pathologist. However, several non-tumor areas shown in Table 1 contain parts of hyperplasia, but not tumors. Samples indicated as “not applicable” (N/A) are due to loss of the non-tumor area in the same sample tissue.

Cell culture

We used two human SCC cell lines (SCCKN and SAS) and fibroblasts. SCCKN, and SAS are established tongue SCC cell lines (15), and the fibroblasts are a normal cell line prepared from a normal lip tissue. SCCKN, SAS, and fibroblasts were maintained in Dulbecco’s Modified Eagle’s Medium (DMEM; Sigma, St. Louis, MO, USA) containing 10% of fetal bovine serum (FBS; JRH Biosciences, Lenexa, KS, USA) at 37°C under 5% CO₂. For apoptosis and cell growth experiments, cells were trypsinized and replating. Treatment was performed when cells completely attached.

Western blot analysis

Cells were washed in phosphate-buffered saline (PBS) and centrifuged. Cell extracts were prepared by lysing the cells in lysis buffer. Samples (10 μg each) were separated by 10% SDS-PAGE. After electrophoresis, the proteins were transferred to a nitrocellulose membrane (Millipore, Bedford, MA, USA), blocked 3 h in TBS with 5% skim milk at room temperature, and then reacted with a primary polyclonal antibody overnight.
The membranes were incubated for 1 h with secondary antibody, and the immune complexes were visualized by using the Immobilon Western (Millipore, Bedford, MA, USA).

**RNA interference approach**

SAS, SCCKN, and fibroblasts were trypsinized and resuspended in DMEM without FBS, and the cells were separated into approximately $1 \times 10^5$ cells for each dish. The Mcl-1 siRNA was purchased from Ambion Applied Biosystems (Tokyo). The sequence of the sense strand of Mcl-1 siRNA is 5'-GGACUUUUAUACCUGUUAUTT-3', and the antisense is 5'-AUAACAGGUAAUAAAAGUCCTG-3'. For the transfection, Mcl-1 siRNA or negative control (Stealth RNAi Negative Control Duplexes; Invitrogen, Inc., Tokyo) solution was added to DMEM medium containing Lipofectamine RNAiMAX and allowed to incubate for 20 min at room temperature to create the transfection mixture. The transfection mixture was then added to the cells at the indicated final concentration of siRNA. At 24 h after the transfection, the medium was changed to DMEM containing 10% FBS. Then, the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay was performed 24 and 48 h after the medium change (14). The cell growth was expressed as the percentage to that of the vehicle control.

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Age/Gender</th>
<th>Differentiation</th>
<th>Mcl-1 expression</th>
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<tr>
<td></td>
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<td>Tumor area</td>
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<tr>
<td>1</td>
<td>64/M</td>
<td>Well-differentiated SCC</td>
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<td>2</td>
<td>64/M</td>
<td>Well-differentiated SCC</td>
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<td>3</td>
<td>47/F</td>
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<td>66/F</td>
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<td>6</td>
<td>71/F</td>
<td>Well-differentiated SCC</td>
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<td>83/F</td>
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<td>64/M</td>
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<tr>
<td>30</td>
<td>52/F</td>
<td>Poorly – moderately differentiated SCC</td>
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**Note:** Expression of Mcl-1 by immunohistochemical staining in the tumor and non-tumor area is scored and expressed as (−) to (+++). SCC: squamous cell carcinoma; N/A: not applicable.
**Apoptosis analysis**

Apoptosis was analyzed by chromatin staining of nuclear condensation with Hoechst 33342 (Sigma) according to the method described previously (14).

**Combination of Mcl-1 siRNA and anti-tumor drug**

Combined treatment of Mcl-1 siRNA with the anti-tumor drug cisplatin was performed. Briefly, after the siRNA treatment, the cells were treated for 48 h with 10 μM of cisplatin (equivalent to 3 μg/ml), which is a slightly effective concentration for cell growth inhibition. Cell growth was measured by MTT assay and expressed as a percentage of the cell growth of non-treated cells.

**Statistical analysis**

All results are expressed as the mean ± S.E.M. Statistical comparisons were made by Student’s t test or Scheffe’s method after analysis of variances (ANOVA). The results were considered significantly different at P<0.05.

**Results**

**Tumor cells in tissues and oral SCC cell lines express Mcl-1 protein**

SCC primary tissues and lymph node metastases were stained using anti-Mcl-1 specific antibody (Table 1 and Fig. 1). Tumor cells in primary tissues and metastatic lymph nodes were positively stained for Mcl-1 (Fig. 1: A and B). On the other hand, Mcl-1 staining was slightly observed in the non-tumor area in the same tissue sections (Fig. 1A, right). Statistically significant difference of the Mcl-1 expression between the tumor and non-tumor area was observed (Fig. 1C). Interestingly, strong expression of Mcl-1 was observed in the well-differentiated tumor tissues (Fig. 1D, upper). In contrast, expression of Mcl-1 in poorly differentiated tumor tissues was much weaker than that in well-differentiated tumor tissues (Fig. 1D, lower and Fig. 1E).

Western blot analysis showed the expression of Mcl-1 in SCC cell lines (SCCKN and SAS) and fibroblasts (Fig. 1F), but Mcl-1 expression in fibroblasts was much lower than those in SCC cell lines. The expression of Mcl-1 was observed in both human SCC tissues and SCC cell lines, but there was little Mcl-1 expression in non-tumor tissue and fibroblasts, indicating the important role of Mcl-1 in SCCs.

**Mcl-1 siRNA inhibits the growth of SCCs**

According to the data of Mcl-1 expression in SCCs, we hypothesized that inhibiting Mcl-1 function might affect the growth of SCCs. To investigate Mcl-1 function on the growth of SCCs, we used the siRNA approach for Mcl-1. Mcl-1 siRNA effectively decreased the Mcl-1 protein level in SCCs and fibroblasts (Fig. 2A). Inhibition of cell growth was observed for both SAS (Fig. 2B) and SCCKN (Fig. 2C) when Mcl-1 was knocked-down by the treatment with Mcl-1 siRNA. In contrast, no inhibition of cell growth was observed in fibroblasts when Mcl-1 was knocked-down (Fig. 2D).

**Mcl-1 siRNA causes apoptotic cell death**

We investigated whether the cell death mediated by Mcl-1 siRNA was caused by apoptosis. The SCCKN and SAS treated with Mcl-1 siRNA showed nuclear condensation fluorescence with Hoechst 33342, which was considered to be a specific morphological change associated with apoptosis (Fig. 3A). We next investigated the activation of caspase-3, an important regulator of apoptosis. The results of the western blotting analysis showed the increased cleavage of caspase-3 by the treatment with siRNA at 24 h (Fig. 3B), also supporting that the inhibition of cell growth was caused by apoptosis.

**Mcl-1 siRNA inhibits phosphorylation of FAK**

We next investigated the mechanisms of cell growth inhibition induced by Mcl-1-siRNA. FAK, a 125-kDa non-receptor tyrosine kinase, is an important regulator of cell survival, invasion, migration, and cell cycle progression. The results of the western blotting analysis showed the decreased expression of phosphorylated FAK by the treatment with Mcl-1 siRNA for 24 h. Consequently, the decreased expression of phosphorylated MEK (p-MEK) and mitogen-activated protein kinase (MAPK, p44/p42MAPK) by the treatment with Mcl-1 siRNA was also observed 24 h after the treatment (Fig. 4: A and B). These data indicate that cell growth suppression by the knockdown of Mcl-1 is mediated through the inhibition of the MAPK pathway.

Inhibition of the ability of cell–extracellular matrix (ECM) adhesion results in the inhibition of the FAK signalling pathways. We, therefore, investigated the potential mechanism involved in the inhibition of cell–ECM adhesion. We investigated the expressions of integrin α5 and β1 because they are well known as major counter adhesion molecules for fibronectin (16, 17). However, no marked differences of the expression of these integrins were observed (data not shown). These results indicated that inhibition of cell–ECM adhesion via alteration of integrin α5 and β1 expressions is not the mechanism for the suppression of the FAK-MAPK pathway by Mcl-1 siRNA.
Combination therapy of Mcl-1 siRNA and anti-tumor drug

Reduction of dosage of anti-tumor drugs for cancer chemotherapy is clinically important to minimize the side effects, although complete tumor cell death is required. Combined treatment of Mcl-1 siRNA (20 nM) with the anti-tumor drug cisplatin (10 μM) drastically inhibited the cell growth in comparison to that in each single treatment (Fig. 5). Similar results were also observed when the combined treatment of Mcl-1 siRNA (20 nM) with another anti-tumor drug, 5-FU, was performed (data not shown). These results indicate that combination therapy of Mcl-1 siRNA and ordinal anti-tumor drugs may be a novel and useful therapy for oral SCCs.

Discussion

Recently, there have been many reports indicating the relationship between Mcl-1 and carcinogenesis. However, the biological role of Mcl-1 on oral squamous cell carcinoma is still unclear. In the present study, we showed that Mcl-1 siRNA inhibited the cell growth of cultured SCCs established from human tongue carcinomas. Using an immunohistochemical approach, our results demonstrated that human oral cancer tissues
Fig. 2. Mcl-1 siRNA inhibits the cell growth of SCCs. A: A Western blot analysis showing Mcl-1 expression in SCCKN, SAS, and fibroblasts that were pre-treated with Mcl-1-siRNA, negative control siRNA, or vehicle (non-siRNA control). Mcl-1-specific siRNA (Mcl-1-siRNA) effectively decreased the Mcl-1 protein level in SCCs and fibroblasts. B, C, and D: Effects of Mcl-1 on the cell growth of SCCKN (B), SAS (C), and fibroblasts (D) treated with Mcl-1 siRNA. Cells were transfected with the siRNA (40 nM) for 24 h and cultured for an additional 48 h, followed by the MTT assay. Each value represents the percentage of cell growth compared with the vehicle (non-siRNA) control from 4 to 8 independent experiments. White column represents the growth of cells transfected with Mcl-1 siRNA and black column represents that of cells transfected with negative control siRNA. Error bars represent S.E.M. Lower panels: typical photographs of transfected SCCKN, SAS, and fibroblast are shown. *$P<0.05$, **$P<0.01$ vs. negative control.
Mcl-1 expression in the well-differentiated tumor area was much stronger than that in the poorly differentiated tumor area. These results may indicate that Mcl-1 plays an important role in the cell growth of well-differentiated SCC rather than that of poorly differentiated SCC. Poorly differentiated tumor cells may utilize other molecules for the prevention of apoptosis rather than Mcl-1. In contrast, well-differentiated SCCs may utilize overexpressed Mcl-1 protein to maintain their proliferation speed, which is comparatively faster. Furthermore, in general and in our clinical cases, the number of patients who were diagnosed with poorly differentiated tumors is much less than the number with well-differentiated or moderately differentiated tumors (17 of 248 cases, 6.9%), and most of the cells in tongue cancer are moderately differentiated or well-

![Fig. 3. Mcl-1 siRNA causes apoptotic cell death. A: Nuclear condensations of the SCCKN (upper) and SAS (lower) cell lines transfected by Mcl-1 siRNA. SCCKN and SAS were treated with Mcl-1 siRNA (40 nM) for 24 h and cultured for 24 h; then the cells were applied to Hoechst 33342 to stain the nuclei. White arrows indicate the nuclear condensations. B: Effects of Mcl-1 siRNA on the activation of caspase-3. Western blot analysis for cleaved caspase-3 was performed. SCCKN and SAS cells were treated with Mcl-1 siRNA (20 nM, 40 nM), negative control siRNA, or vehicle (Veh). Samples were collected 24 h after the treatment. GAPDH was used to evaluate equivalent loading.]

![Fig. 4. Mcl-1 siRNA inhibits the phosphorylation of FAK. A and B: Effects of Mcl-1 siRNA on phosphorylation of FAK, MAPK, and MEK. Western blot analysis for phosphorylated FAK (Tyr 925), MAPK (p44/p42 MAP kinase, Thr202/Tyr204), and MEK1/2 (Ser217/221). SCCKN and SAS cells were treated with Mcl-1 siRNA (20, 40, and 80 nM), negative control siRNA, or vehicle (Veh). Samples were collected for 24 h after the treatment. GAPDH was used to evaluate equivalent loading.]

expressed higher levels of Mcl-1 than non-tumor tongue tissues. Similar results were also observed on the cultured SCC cell lines such as SAS and SCCKN, although weak expression in fibroblasts was observed. Moreover, Mcl-1 expression in the well-differentiated...
differentiated cells. Therefore, investigation of Mcl-1 expression in tongue cancer is important, and over-expressed Mcl-1 in well-differentiated SCCs may be a good target for tumor therapy.

A recent study showed that Mcl-1 and Bel-2 are expressed at different stages of differentiation in numerous normal tissues (18). These two proteins commonly appeared in gradients with opposing directions, such that the expression of Bel-2 tended to be higher in the less-differentiated cells, whereas Mcl-1 was more intense in the differentiated cells (18, 19). In our present study, well-differentiated tumors showed higher Mcl-1 expression than poorly differentiated tumors. In contrast, immunochemical detection showed that there was very low expression of Bel-2 in both well-differentiated and poorly differentiated tongue carcinomas (data not shown). Together with these findings, the present results indicated that Mcl-1 is important for the survival of well-differentiated SCCs.

Next, we investigated whether Mcl-1 siRNA was a potential target for tumor therapy of SCCs. In the present study, we showed that Mcl-1 siRNA inhibited the cell growth of cultured SCCs established from human tongue carcinomas. Furthermore, the inhibition of cell growth of SCCs by Mcl-1 siRNA was, in part, due to apoptosis because chromatin staining with Hoechst 33342 revealed nuclear condensation. In SCC lines, visible differences were observed at 48 h after the treatment, but not in fibroblasts. These results suggest that Mcl-1 siRNA causes the inhibition of tumor cell growth, but not that of normal cells.

In our present study, the inhibition of phosphorylation of FAK in SCCs treated with Mcl-1 siRNA was also observed. FAK is a 125-kDa non-receptor tyrosine kinase and an important regulator of cell survival, invasion, migration, and cell cycle progression (20, 21). The overexpression of FAK was observed in a number of human malignant cells, with the degree of over-expression correlating with greater aggressiveness (22). FAK is functionally important in transducing intracellular messages that are associated with growth factor signalling (23). The intracellular messages link p-FAK at Tyr\textsuperscript{925} to signalling pathways that modify the cytoskeleton and activate MAPK cascades. In the present study, inhibitions of the MEK and MAPK phosphorylation after the inhibition of FAK-phosphorylation by Mcl-1 siRNA were observed. Our results indicate that the mechanisms of the inhibition of cell growth by Mcl-1 siRNA are in part due to the inhibition of the FAK-MAPK pathway.

Malignant cells are able to resist apoptosis to varying degrees, and this property has been proposed to contribute to tumorigenesis and metastasis (24). It is well known that apoptosis and inhibition of cell growth in SCC cell lines are induced by anoikis (apoptosis resulting from loss of cell–matrix interactions), and p-FAK is reported to be involved in anoikis (14, 25). The activation of the FAK signalling pathway requires cell–ECM adhesion, which is inhibited by decrease in integrin \(\alpha_5\) and \(\beta_1\) expression. Integrin \(\alpha_5\) and \(\beta_1\) are well known as the counter receptor for fibronectin. Under the present experimental conditions, we confirmed that integrin \(\alpha_5\) and \(\beta_1\) expression was not affected by the treatment with Mcl-1 siRNA, indicating no inhibition of cell–ECM adhesion. In addition, morphological differences such as cell detachment as an index of anoikis were not observed. These results may suggest that Mcl-1 siRNA directly inhibits FAK phosphorylation followed by the inhibition of the MAPK pathway rather than decreasing integrin expression (14, 25).

In the present study, we showed two major potential mechanisms for how Mcl-1 siRNA inhibits SCC growth: one was the suppression of cell proliferation via inhibition of the MAPK pathway, and the other was induction of apoptosis. According to our data, the suppression of proliferation rate by Mcl-1 siRNA in comparison to the
negative-siRNA control was drastic, but the total cell number was not diminished by the treatment with Mcl-1 siRNA. In addition, the percentage of apoptotic cells upon treatment with Mcl-1 siRNA was about 10%. Therefore, contribution of the MAPK pathway inhibition on the mechanisms of cell growth inhibition by Mcl-1 siRNA is considered to be much larger than that of the induction of apoptosis. Further investigations are required to clarify the detailed mechanisms.

It was reported that decrease in Mcl-1 level increased the sensitivity of cancer cells to apoptosis induced by chemotherapy and radiotherapy (12, 13). In contrast to the previous reports, a recent study indicated that Mcl-1–expressing tumor cells are more sensitive to radiotherapy and chemotherapy than Mcl-1–deficient tumor cells (26). Namely, two discrepant hypotheses are raised. At least, the Mcl-1 level in tumor cells seems to be closely related to the sensitivity for chemotherapy and radiation. In fact, in our present study, combined treatment of Mcl-1 siRNA with anti-tumor drugs, cisplatin and 5-FU, drastically inhibited the cell growth in comparison to that in each single treatment. Cisplatin is extensively characterized as a DNA damaging agent and the cytotoxicity of cisplatin is attributed to the ability to form inter and intra-strand nuclear DNA crosslinks (27, 28). In contrast, inhibition of cell growth by Mcl-1 siRNA presented in our study was mainly due to the inhibition of the MAPK pathway. Therefore, the mechanisms between Mcl-1 siRNA and cisplatin on tumor cell growth inhibition are different. This difference of mechanisms between Mcl-1 siRNA and cisplatin may lead to the synergistic effect of cisplatin and siRNA on the inhibition of tumor cell growth. Our results also indicate that decrease in Mcl-1 level in SCCs that strongly express Mcl-1 increases the sensitivity to chemotherapy. Further experiments are required to clarify the correlation of Mcl-1 with the sensitivity for radiotherapy and chemotherapy and to delineate the precise contributions and mechanisms of Mcl-1 inhibition in terms of tumor transformation, growth, survival, and metastasis. At least, our results indicate that suppression of Mcl-1 itself by the siRNA technique is enough to cause the cell death of SCCs, and local administration of siRNA for Mcl-1 combined with ordinal anti-tumor drugs might be a useful therapy to reduce the dosage of anti-tumor drugs in the future because most of the cells in tongue cancer are moderately differentiated or well-differentiated cells that strongly express Mcl-1.

In summary, we showed the expression of Mcl-1 in tumor cells of human primary SCC and cultured SCC cell lines and suggested a potentially important role for Mcl-1 in the treatment of human oral SCC cases.

Acknowledgments

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