CD147 promotes invasion and MMP-9 expression through MEK signaling and predicts poor prognosis in hypopharyngeal squamous cell carcinoma

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Abstract

Background. Hypopharyngeal cancer is one of the most frequent head and neck cancers and is associated with a poor prognosis because of recurrence and metastases. Therefore, there is a need to improve the prognosis, which requires the identification of prognostic factors and elucidation of the mechanisms involved in tumor progression. Accumulated evidence has demonstrated that cluster of differentiation 147 (CD147) is strongly expressed in malignant tumors, including head and neck squamous cell carcinoma (HNSCC), and contributes to tumor progression.

Objectives. To investigate CD147-induced signaling pathways in HNSCC cells to evaluate the mechanisms of tumor progression mediated by CD147, and the association between CD147 expression in tumors and the survival rate of hypopharyngeal cancer patients.

Material and methods. To determine the downstream signaling of CD147 in HNSCC, expression levels of phosphorylated AKT1, MEK1, p38 MAPK, STAT3, and NF-κB were evaluated using enzyme-linked immunosorbent assay (ELISA) in FaDu, a hypopharyngeal cell line, exposed to cyclophilin A, a CD147 ligand.

Results. We found that hypopharyngeal cancer patients expressing CD147 showed a poor five-year overall survival (OS) of 11.1% compared with those without CD147 expression (43.0%) ($p = 0.02$). We confirmed that the expression of phosphorylated MEK and matrix metalloproteinase-9 (MMP-9), as well as cell invasion ability, were enhanced in hypopharyngeal cancer cells. In addition, this increased cell infiltration and enhancement of MMP-9 expression induced by CD147 were abolished by a MEK inhibitor.

Conclusions. These results suggest that CD147 can be a predictor of a poor prognosis, and that a CD147-induced MEK-mediated intracellular signaling pathway plays a crucial role in tumor progression in hypopharyngeal carcinoma.

Key words: invasion, MMP, hypopharyngeal cancer, CD147, MEK

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Introduction

Hypopharyngeal cancer is among the most frequent head and neck cancers. Multidisciplinary treatment is administered according to the stage of the disease, but the prognosis is poor due to recurrence and metastasis.[1] Developing more efficient treatments is essential to improve the prognosis. For this purpose, it is necessary to elucidate the mechanisms of tumor progression.

In recent years, key factors involved in cancer progression have been identified.[2] The elucidation of the mechanisms involved in tumor progression in head and neck cancer, including hypopharyngeal cancer,[3] is a topic of high interest. Furthermore, new therapeutic targets, such as downstream components of receptor signaling cascades, intracellular tyrosine kinases, PI3K, and STAT3, have been identified.[4]

Cluster of differentiation 147 (CD147), also known as extracellular matrix metalloproteinase inducer (EMMPRIN), is a member of the immunoglobulin superfamily that is highly expressed in cancer cells.[5] CD147 induces the production of matrix metalloproteinases (MMPs), which have many physiological effects, including cell proliferation and extracellular matrix (ECM) degradation. Therefore, CD147 plays an important role in tumorigenesis. CD147 contributes to a variety of malignant phenotypes, including head and neck squamous cell carcinoma (HNSCC).[6] In addition, it has been reported that CD147 is a predictor of a poor prognosis in HNSCC patients.[7] We also previously reported that CD147 expression is associated with cervical lymph node metastasis in patients with tongue squamous cell carcinoma (SCC).[8]

Studies have attempted to elucidate the mechanisms underlying CD147-induced tumorigenesis in various types of cancer, and the number of studies on the contribution of CD147 to the progression of HNSCC is increasing.[9] However, its detailed mechanism has not been fully elucidated yet.

Intracellular signal transduction is an important cell regulatory mechanism.[10] The importance of signal transduction during cancer progression is also widely recognized, and research on this topic is continuously becoming more intensive.[11,12] In recent years, intracellular signals have been elucidated in many carcinomas, including head and neck cancers, suggesting their potential as therapeutic targets.[13] In particular, CD147-induced signaling pathways have been investigated in various carcinomas, and the importance of pathways involving Smad, mTOR and STAT3 has been reported.[14–16]

The involvement of MAPK/ERK signaling in HNSCC has also been reported.[17] However, the role of CD147 in HNSCC tumorigenesis and the underlying mechanisms, including cell signaling pathways, in hypopharyngeal cancer are not fully understood. The purpose of this study was to investigate the mechanisms of tumor progression mediated by CD147 and clinical features of CD147 in hypopharyngeal cancer.

Material and methods

Patients

In this study, we examined 19 patients with pathologically confirmed SCC in their hypopharynx who underwent surgery at the Department of Otorhinolaryngology & Head and Neck Surgery at the Akita University Graduate School of Medicine (Japan) between 2005 and 2014. Patient consent was not deemed necessary because the patient information was obtained from a pathology archive in which patient identification was anonymized and de-identified prior to analysis. All the patients were followed up for at least 60 months or until they died after surgery. The tumors were classified according to the 2002 Union for International Cancer Control staging system (Table 1).[18]

Table 1. Patients' characteristics and pathological findings

Variable	Number of patients
Sex	
male	17
female	2
Age [years]	
median	62
range	49–80
Stage	
III	1
IV	18
CD147 index	
0	8
2	2
4	9
Follow-up [months]	
median	68
range	6–91
Prognosis	
survived	6
died from hypopharyngeal cancer	10
died because of factors other than hypopharyngeal cancer	3
Differentiation type	
poor	5
moderate	10
well	4
Vessel invasion	
positive	11
negative	8
Lymph vessel invasion	
positive	12
negative	7
Perineural invasion	
positive	9
negative	10

CD147 – cluster of differentiation 147.

Immunohistochemistry and classification of pathological findings

Excised primary tumor specimens were fixed with 10% neutral-buffered formalin, and consecutive sections were cut every 5 mm; 4-µm thick tissue sections were obtained. The sections were stained with hematoxylin and eosin (H&E), and the section containing the invasive tumor front was selected for further analysis. The polyclonal anti-CD147 antibody (Santa Cruz Biotechnology, Santa Cruz, USA) was used as the primary antibody for immunohistochemical staining. In brief, 4-µm thick sections were deparaffinized and initially autoclaved for 15 min at 121°C. Sections were then blocked with 0.3% hydrogen peroxide in methanol for 30 min at room temperature and with 10% normal rabbit serum/Tris (Vector Laboratories Inc., Burlingame, USA) for 30 min at room temperature. All the sections were kept overnight at 4°C in phosphate-buffered saline (PBS) containing the rabbit anti-human CD147 polyclonal antibody (dilution: 1:200), followed by a 1 h incubation with biotinylated anti-rabbit IgG (ready-to-use dilution, cat. No. ab64256; Abcam, Cambridge, UK) at room temperature. The sections were washed with PBS and protein expression was detected with the Vectastain avidin-biotin complex kit (Vector Laboratories Inc.) according to the manufacturer's instructions, and then reacted with diaminobenzidine (Nacalai Tesque Inc., Kyoto, Japan) for 3–5 min at room temperature.

A pathologist and surgeon evaluated the CD147 immunohistochemical staining. CD147 expression in a tumor was scored according to the staining strength and intensity at ×200 magnification using light microscopy. The areas of CD147 staining received scores of 0 if <10% of cells in the tumor nest were positive; scores of 1 if ≥10% but <50% of cells in the tumor were positive; and scores of 2 if ≥50% of cells of the tumor were positive. CD147 staining intensity was also scored from 0 to 2 (negative, weak or strong staining), and a CD147 index (range: 0–4) was calculated as the CD147-positive area score (0-2) × CD147 intensity score (0–2). An index of 4 was classified as positive.

In addition, H&E-stained samples were examined to determine the SCC differentiation type and the presence or absence of lymphovascular, vascular and perineural invasion. Samples were classified according to the pathological findings as poorly, moderately or well-differentiated, and assessed for the presence or absence of lymphovascular, vascular and/or perineural invasion.

Cells and cell culture

FaDu, a cell line derived from human SCC of the hypopharynx that expresses CD147,[19] was a kind gift from the Department of Cell Biology and Morphology at the Akita University Graduate School of Medicine and was used for in vitro studies. Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated fetal bovine serum (FBS) and incubated at 37°C in the presence of 5% CO_2. For the stimulation experiments, FaDu cells were pre-incubated with serum-free DMEM and then incubated with serum-free medium containing cyclophilin A (CypA; Sigma-Aldrich, St. Louis, USA). A MEK inhibitor, U0126 (Wako Pure Chemical Industries Ltd., Osaka, Japan), was used for the MEK inhibition studies.

Matrigel invasion assay

We evaluated cell invasiveness in vitro using Matrigel-coated semipermeable-modified Boyden inserts with a pore size of 8 µm (Becton Dickinson & Co., Franklin Lakes, USA). In addition, FaDu cells were plated at a density of 2.5 × 104 cells/insert in serum-free medium with or without CypA (400 ng/mL). Notably, the lower chamber contained DMEM + 10% FBS and served as a chemoattractant. Meanwhile, we plated cells in 96-well plates to serve as loading controls. After a 48-hour treatment at 37°C in a 5% CO_2 incubator, we removed the cells in the insert by wiping gently with a cotton swab. Next, the cells on the reverse side of the insert were fixed and stained using Diff-Quick (Sysmex, Kobe, Japan) according to the manufacturer's instructions. We counted the invading cells in 4 representative fields using light microscopy at ×200 magnification, and evaluated the mean ± standard deviation (SD) from 3 independent experiments. Additionally, the cells plated in 96-well plates as loading controls were subjected to 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assays, which meant that cell invasion ability was evaluated without the effects of increases in the number of cells across the groups.

Gelatin zymography

FaDu cells were cultured overnight using ordinary serum-containing DMEM, and then washed with serum-free DMEM and further cultured using serum-free DMEM with/without 400 ng/mL of CypA for 48 h. This was followed by detection of gelatinolytic activity in the conditioned media using gelatin zymography. In brief, the conditioned medium was resolved with SDS-PAGE under non-reducing conditions using 7.5% separating gel containing 0.3 mg/mL gelatin. Then, the gels were washed twice in 2.5% (w/v) Triton X-100 for 30 min at room temperature to remove the SDS, then incubated in reaction buffer containing 50 mM Tris–HCl, pH 7.6, 5 mM $CaCl_2$, and 2.5% Triton X-100 for 24 h at 37°C, followed by staining with 2.5% (w/v) Coomassie brilliant blue R- 250 in 30% (v/v) methanol and 10% (v/v) acetic acid. After de-staining with 30% methanol and 10% acetic acid, gelatinolytic activities on the gel were detected as clear bands on a blue background of undigested gelatin.

siRNA and siRNA transfection

CD147 siGENOME siRNA (Dharmacon RNA Technologies, Lafayette, USA) was transfected into FaDu cells for CD147 silencing. We used siGENOME non-targeting siRNA as control. The siRNA transfections were performed using Lipofectamine 2000 (Life Technologies Inc., Carlsbad, USA). In brief, 1.8×10^5 FaDu cells were plated on a six-well plate. After a 24-hour incubation in complete media, the cells were transfected with 200 pmol of CD147 siRNA or non-targeting control siRNA. The transfection medium was replaced with complete media after 4 h of transfection.

Semi-quantitative determination of AKT, Stat3, p38 MAPK, MEK1, and NF-κB phosphorylation status

PathScan® Signaling Nodes Multi-Target Sandwich ELISA Kit No. 7272 was purchased from Cell Signaling Technology, Inc. (Danvers, USA). This solid phase sandwich enzyme-linked immunosorbent assay (ELISA) combines the reagents necessary to detect endogenous levels of AKT1, phospho-AKT1 (Ser473), phospho-MEK1 (Ser217/221), phospho-p38 MAPK (Thr180/Tyr182), phospho-Stat3 (Tyr705), and phospho-NF-κB p65 (Ser536). FaDu cells (75–80% confluent) were starved overnight and then cultured in the absence or presence of 200 ng/mL of CypA in low-serum (0.1% FBS)-containing culture medium for 48 h. The cells were washed twice with cold PBS and then lysed in buffer (20 mM Tris pH 7.5, 150 mM NaCl, 1 mM EDTA, 1 mM EGTA, 1% Triton® X-100, 2.5 mM sodium pyrophosphate, 1 mM ß-glycerolphosphate, 1 mM Na3VO4, 1 µg/mL leupeptin, 1 mM phenylmethylsulfonylfluoride), and a complete protease inhibitor cocktail from Sigma-Chemicals (St. Louis, USA), for 30 min on ice. The lysates were cleared by centrifugation in an Eppendorff tube (15 min at 14,000 × g, 4°C). The protein content was determined against a standardized control using a commercially available protein assay kit (Pierce Biotechnology Inc., Rockford, USA). Differential phosphorylation of AKT1, phospho-AKT, phosphoMEK1, phospho-p38 MAPK, phospho-Stat3, and phospho-NF-κB p65 was measured in accordance with the manufacturer's instructions. Briefly, after incubation with cell lysates at a protein concentration of 0.5 mg/mL, the target phospho-protein was captured using the antibody coated onto the microwells. Following extensive washing, a detection antibody was added to detect the captured target phospho-protein. An HRP-linked secondary antibody was then used to recognize the bound detection antibody. The HRP substrate TMB was added to develop color. The magnitude of absorbance (measured at 450 nm) for the developed color is proportional to the quantity of bound target protein.

Statistical analysis

Statistical analyses were performed using Statcel 3 (OMS Publishing, Tokorozawa, Japan). The Wilcoxon–Mann–Whitney two-sided exact test was used to assess the statistical significance of the differences in MMP-9 expression and invasion studies. Fisher's exact test was used to determine the relationship between CD147 expression and the differentiation type, lymphatic invasion, vascular invasion, and perineural invasion. A value of $p < 0.05$ was considered to indicate a statistically significant difference. The difference in survival between patients stratified by CD147 levels was evaluated with a Kaplan–Meier survival analysis.

Results

Patient data and pathological findings

As presented in Table 1, 19 patients (17 men and 2 women) with hypopharyngeal SCC were included in the present study. All the patients presented with stage III or IV (stage III: 1 patient, stage IV: 18 patients). The patients ranged in age from 49 to 80 years (median: 62 years). During the follow-up period (median: 68 months, range: 6–91 months), 10 patients died from hypopharyngeal cancer and 6 survived. Three patients died because of factors other than hypopharyngeal cancer.

When immunohistochemistry was used to measure CD147 levels in tumor tissues, 8 samples were given a score of 0; 2 were given a score of 2; and 9 were given a score of 4. Accordingly, the 9 cases scored as 4 were defined as positive, and the remaining 11 cases were considered negative for CD147 expression.

Regarding the other histopathological findings, 5 cases were poorly differentiated, 10 cases were moderately differentiated and 4 cases were well-differentiated. In addition, 11 exhibited vascular invasion, 12 lymphovascular invasion and 9 perineural invasion.

The patient characteristics and pathological findings are summarized in Table 1.

CD147 expression in primary tumors is an independent post-surgical predictor of a poor prognosis

We then investigated the association between CD147 levels and the survival rate in hypopharyngeal cancer patients using the Kaplan–Meier method. We found that hypopharyngeal cancer patients with positive CD147 expression showed a lower five-year overall survival rate (OS) at 60 months (11.1%) compared with patients with negative CD147 expression (43.0%) ($p = 0.023$; Fig. 1). Therefore, higher CD147 expression is associated with a poor prognosis in hypopharyngeal cancer patients. In contrast, there were no significant differences in the survival rates in terms of SCC

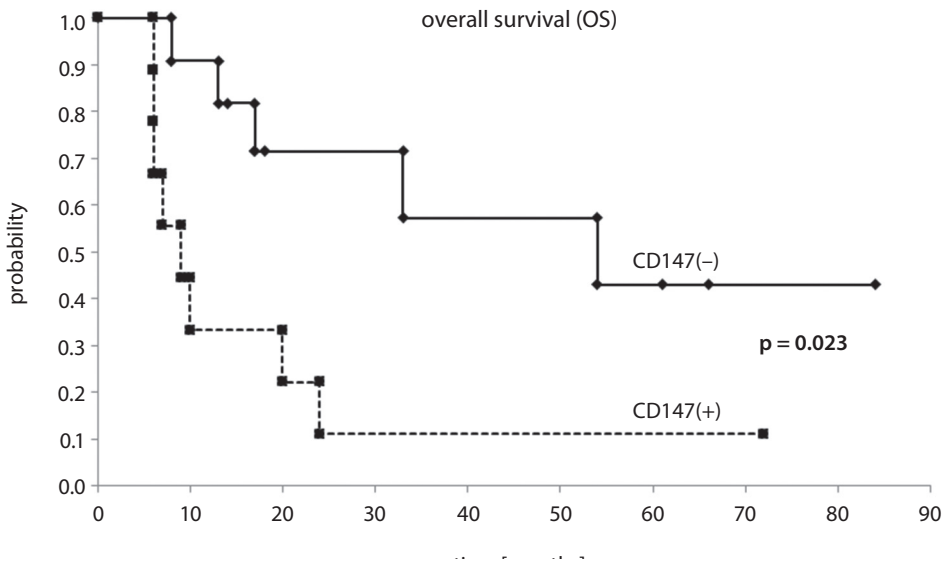

Fig. 1. Overall survival (OS) stratified according to CD147 status among hypopharyngeal cancer patients (log-rank test, p = 0.023)

Table 2. The associations between histopathological findings and CD147 expression

Characteristics		CD147		p-value
		positive	negative	
Differentiation type	moderate/poor	7	8	0.667
	well	2	2	
Vascular invasion	positive	7	4	0.115
	negative	2	6	
Lymphovascular invasion	positive	7	5	0.220
	negative	2	5	
Perineural invasion	positive	3	1	0.249
	negative	6	9	

CD147 – cluster of differentiation 147.

differentiation type and the presence or absence of lymphovascular, vascular and perineural invasion (data not shown).

In addition, we investigated the association of CD147 expression and representative pathological findings, including SCC differentiation type and the presence or absence of lymphovascular, vascular and perineural invasion. However, there were no significant correlations between CD147 expression and the pathological findings (Table 2). These results suggest that CD147 may be an independent prognostic factor.

CypA-CD147 interactions upregulate the expression of phosphorylated MEK in FaDu cells

We assessed the activation status of convergence points and key regulatory proteins in several signaling pathways controlling cellular events that contribute to tumor progression. We used the PathScan® Signaling Nodes Multi-Target Sandwich ELISA kit (Cell Signaling Technology, Inc.) to simultaneously assess in a semi-quantitative manner the endogenous levels of AKT1, phospho-AKT1 (Ser473), phospho-MEK1 (Ser217/221), phospho-p38 MAPK (Thr180/Tyr182), phospho-Stat3 (Tyr705), and phospho-NF-κB p65 (Ser536) in FaDu cells.

Phosphorylation is the most common mechanism of regulating protein function and transmitting signals throughout the cell.[20] Therefore, the observation of a phosphorylated signaling factor implies activation of the pathway in which the factor is involved.

To assess the signaling pathways associated with CD147, FaDu cells were cultured with 400 ng/mL CypA. We previously reported that CypA induces tumorigenicity by interaction with CD147 in FaDu cells.[21] The findings revealed that among the signaling nodes evaluated, only the protein level of phospho-MEK was upregulated in the presence of CypA.

We then used siRNA to downregulate CD147 before culturing the FaDu cells (Fig. 2A) in the presence or absence of CypA. By silencing CD147, if the protein expression levels of signaling nodes are changed by CypA, it is possible to validate whether CD147 mediates this alteration. We found that CypA induced significant increase in phospho-MEK expression in non-targeting siRNA-treated cells, but this increase was not observed following CD147 knockdown (Fig. 2B). These results suggest that MEK is an important signaling pathway related to CD147 in hypopharyngeal SCC.

MEK mediates CypA-induced cell invasion and MMP-9 expression in FaDu cells

As tumor cell invasion is an important stage in metastasis, several studies have focused on developing methods to control invasion as a target for clinical tumor suppression.[22] In addition, it has been reported that MMPs, especially gelatinases MMP-9 and MMP-2, play an important role in tumor invasion and metastasis through degradation of the basement membrane.[23] To evaluate invasion, FaDu

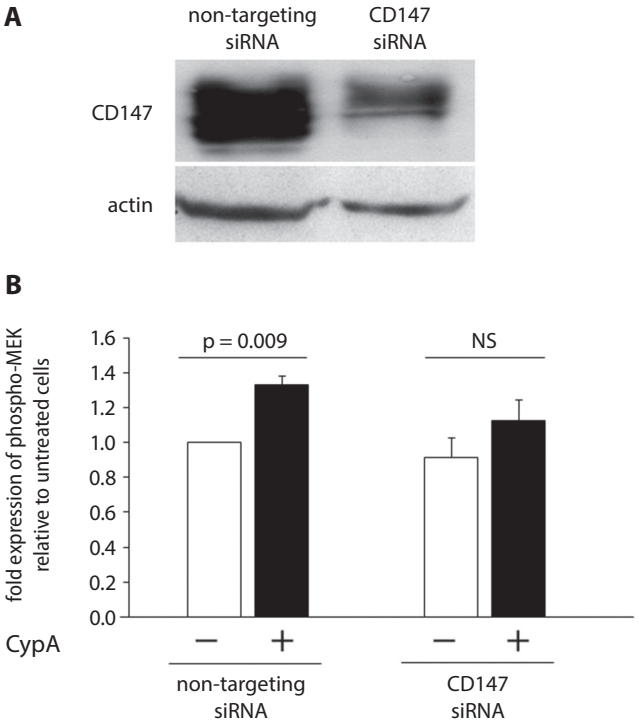

Fig. 2. A. CypA-CD147 interactions upregulate the expression of phosphorylated MEK in hypopharyngeal cancer cells. FaDu cells were transfected with non-targeting siRNA or CD147 siRNA. CD147 expression was analyzed with western blotting. CD147 expression was successfully downregulated 48 h after siRNA transfection; B. FaDu cells were then cultured with or without 200 ng/mL of CypA. The expression of phospho-MEK was examined using ELISA. The experiment was repeated 5 times, and similar results were observed for each replication. The results are expressed as fold change relative to untreated FaDu cells. A significant increase in phospho-MEK induced by CypA was observed during non-targeting siRNA treatment; however, this significance was not observed during CD147 knockdown

NS – not significant ($p > 0.05$); siRNA – small interfering RNA; CypA – cyclophilin A.

cells were seeded into Matrigel-coated invasion chambers and stimulated with CypA. The expression levels of gelatinases were also detected using gelatin zymography in FaDu cells treated with or without CypA. It was revealed that cell invasion was increased in the presence of CypA. In addition, only the expression of MMP-9 was detected, indicating that MMP-9 expression was increased in the presence of CypA.

To determine whether MEK signaling mediates these changes in invasion and MMP expression by CypA, we treated FaDu cells with U0126, a specific MEK inhibitor, in the presence or absence of CypA. We found a significant increase in cell invasion and upregulation of MMP-9 expression by CypA in the absence of U0126. However, these increments were not observed in the presence of U0126 (Fig. 3,4). These results suggest that the MEK signaling pathway mediates CD147 tumorigenesis in hypopharyngeal SCC.

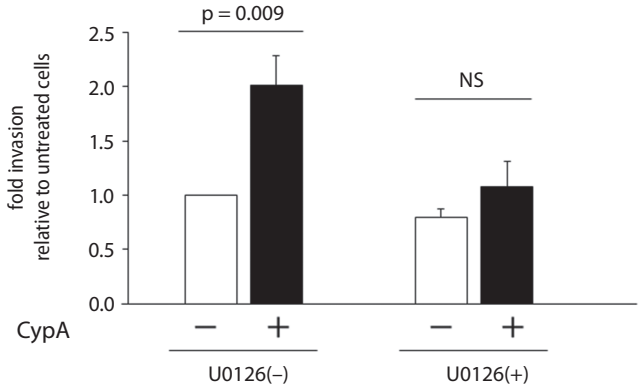

Fig. 3. MEK mediates CypA-induced cell invasion in hypopharyngeal cancer cells. Cell invasiveness was evaluated in vitro using a Matrigel invasion assay. FaDu cells were plated in the inserts in serum-free medium with or without 200 ng/mL of CypA, 10 nM of U0126 (a specific MEK inhibitor), or a combination of CypA and U0126. The number of invading cells in each insert was determined, and means ± standard error (SE) were calculated from 5 independent experiments. The number of invading cells significantly increased following stimulation with CypA compared with untreated cells, and this increase was attenuated by U0126

NS – not significant ($p > 0.05$); CypA – cyclophilin A.

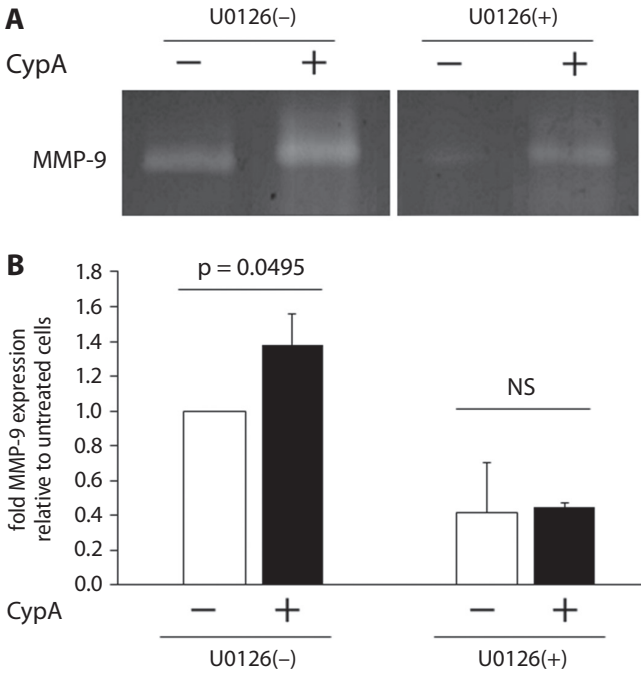

Fig. 4. MEK mediates CypA-induced expression of MMP-9 in hypopharyngeal cancer cells. MMP-9 expression was evaluated using gelatin zymography. FaDu cells were cultured in serum-free medium with or without 200 ng/mL of CypA, 10 nM of U0126 (a specific MEK inhibitor), or a combination of CypA and U0126. A. A representative result of gelatin zymography used to detect MMP-9 in the culture media of FaDu cells; B. Densitometry analysis performed on the gelatin zymography results of MMP-9 levels relative to MMP-9 in untreated FaDu cells. Means ± standard error (SE) were calculated from 3 independent experiments. The expression of MMP-9 significantly increased following stimulation with CypA compared with untreated cells, and this increase was attenuated by U0126

NS – not significant ($p > 0.05$); CypA – cyclophilin A.

Discussion

It is ideal to improve the effectiveness of cancer treatments while suppressing toxic side effects. For that purpose, it is important to determine the characteristics and identify targets of cancer. To this end, the most important issue is to elucidate the factors involved in the development of each type of cancer.[24]

Hypopharyngeal cancer is one of the most frequent head and neck cancers and has been shown to have a direct impact on the patients' quality of life, such as causing difficulties with swallowing and phonation. It is associated with a poor prognosis, and there is an essential need to improve therapeutic effects and to reduce unwanted side effects.[1,25]

To improve the therapeutic effects of treatments for malignant tumors, it is necessary to first understand the characteristics of the carcinoma and to take measures against the factors. Various studies have been conducted on head and neck cancer, and based on the results, attempts have been made to predict the prognosis and select the optimal treatment.[26]

In head and neck cancers, clinical findings like tumor size and cervical lymph node metastasis have been studied as predictors of the prognosis.[27] In addition to these clinical prognostic factors, studies on new biomarkers, like human papillomavirus infection or EGFR expression, have progressed in recent years. Currently, these factors are used to help predict the prognosis and are considered to be actual therapeutic targets.[13,28]

CD147 is known to be associated with prognosis in various malignant tumors. Targeting CD147 has been shown to be effective, and has great significance in clinical practice.[29] In this study, we found that CD147 expression in hypopharyngeal carcinoma was related to the prognosis. These results suggest that CD147 may be an efficient therapeutic target in hypopharyngeal cancer.

While CD147 clearly affects the prognosis, in order to apply these findings to the development of therapeutic methods, it is necessary to elucidate the mechanisms that promote tumor progression. Originally, CD147 was reported as an inducer of MMPs during physiological functions such as wound healing, but this action has also been shown to play an important role in cancer progression.[30] In cancer, it is well-established that CD147 is involved in invasion and metastasis mainly by MMP-mediated ECM and basement membrane degradation. It also regulates cell proliferation, drug resistance and epithelial-to-mesenchymal transition in many carcinomas, including head and neck cancer.[9,21]

The MMPs, a family of zinc-dependent proteinases, are capable of degrading a variety of ECM components and have various physiological functions in normal tissues.[30] Furthermore, the degradation of ECM components by MMPs is a determining step in tumor cell invasion and metastasis.[31] In particular, MMP-2 and -9 (gelatinase A and B) are able to degrade type IV collagen in the basement membrane to promote tumor progression.[23,32] It has been reported that MMP-9 is involved in the cell invasion of hypopharyngeal carcinoma.[33] Therefore, the result of the present study showing the involvement of MMP-9 expression in hypopharyngeal cancer cells is important for elucidating the key factors that regulate hypopharyngeal cancer progression.

Intracellular signal transduction is very important for normal cell functions, and understanding these pathways is indispensable for elucidating the behavior of cancer cells.[34] To date, key signaling pathways have been identified in various carcinomas,[35] and the therapeutic effects of targeting them have been reported.[36] Signal transduction pathways in head and neck cancers have also been elucidated, and the therapeutic effects of targeting them have been shown.[37] However, there is limited research on hypopharyngeal cancer. In this study, certain signaling pathways, including MEK, were suggested as signals activated by CD147 in a hypopharyngeal cell line.

MEK is a key factor in the Ras/Raf/MEK/ERK (MAPK) signaling pathway. This MAPK cascade forms a complex signaling network that controls a wide variety of cellular processes, such as cell proliferation, growth, differentiation, transformation, and apoptosis.[38] Dysregulation of key mediators of the MAPK pathway has been implicated as a driver of tumorigenesis and contributes to the ability of cancer cells to be independent of mitogen signals, produce growth factors, avoid apoptosis, acquire a sensitivity to antiproliferative signals, metastasize, and gain angiogenic potential.[39] Much effort has been directed toward the development of inhibitors of this pathway, assuming that mutations in the MAPK pathway contribute to tumor-promoting processes.[38] Among them, MEK has been shown to be a potential therapeutic target for some solid cancers, such as prostate cancer, and inhibitors are being developed.[40]

Conclusions

In summary, studies using clinical specimens have shown that CD147 expression in hypopharyngeal carcinoma is a predictor of a poor prognosis. In this study, CD147 expression in hypopharyngeal cancer cells was stimulated using CypA, a CD147 ligand. As a result, it was confirmed that cell invasion ability, MMP-9 expression and phosphorylated MEK levels were enhanced in hypopharyngeal cancer cells. In addition, cell infiltration and the enhancement of MMP-9 expression induced by CD147 were abolished by a MEK inhibitor.

Clinical studies suggest that CD147 may be associated with short OS in hypopharyngeal carcinoma patients. However, the number of patients analyzed in this study was small. Further studies of the prognostic impact of CD147 should be performed with a larger sample size. However, the in vitro results indicate that the CD147-induced MEK-mediated intracellular signaling pathway plays an important role in tumor progression in hypopharyngeal carcinoma, and inhibition of these factors may help to control

hypopharyngeal tumor progression and improve the prognosis. Further studies on CD147 and MEK signaling pathways are expected in the future.

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