Implications of Cortactin expression in pathogenesis of oral squamous cell carcinoma

Min-Ji Kim

The Graduate School
Yonsei University
Department of Dentistry

Implications of Cortactin expression in pathogenesis of oral squamous cell carcinoma

A Dissertation Submitted
to the Department of Dentistry
and the Graduate School of Yonsei University
in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy in Dental Science

Min-Ji Kim

June 2024

This certifies that the doctoral dissertation of
Min-Ji Kim is approved.

Thesis Supervisor : Woong Nam

In-Ho Cha

Hyung Jun Kim

Dongwook Kim

Xianglan Zhang

The Graduate School
Yonsei University
June 2024

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

ABSTRACT

Implications of Cortactin expression in pathogenesis of oral squamous cell carcinoma

Min-Ji Kim

Department of Dentistry
The Graduate School, Yonsei University

(Directed by Professor Woong Nam)

Early diagnosis and treatment of oral squamous cell cancer are common challenges in the field of oral surgery. Cortactin(CTTN), a major structure protein of invadopodia in cancer tissues, can mediate various changes in the biological behavior of cancer cells. However, the related molecular mechanisms are largely unknown.

In our study, the implications of expression of cortactin on biological behavior of OSCC cells were investigated in vitro. Moreover, the clinicopathological importance of CTTN expression was also investigated in OSCC cohort.

Decreased proliferation, motility, and invasion ability were found in CTTN knockdown OSCC cells than related control cells. Moreover, increased apoptosis was found in CTTN knockdown OSCC cells than in related control cells. Furthermore, IL-1β expression was significantly decreased after CTTN knockdown in OSCC cells than control cells. Supportively, significant associations were found between IL-1β and CTTN expression in OSCC cohort. In OSCC cohort, CTTN overexpression showed positive correlation with lymph node metastasis, desmoplastic reaction, and poor prognosis.

In conclusion, CTTN exhibits oncogenic activity in OSCC, and these results further support CTTN as a novel prognostic molecular biomarker in OSCC patients.

Keywords: CTTN, Lymph node metastasis, Desmoplasia, Molecular biomarker, OSCC

Implications of Cortactin expression in pathogenesis of oral squamous cell carcinoma

Min-Ji Kim

Department of Dentistry
The Graduate School, Yonsei University

(Directed by Professor Woong Nam)

1. Introduction

In South Asia, which includes India and Sri Lanka, oral squamous cell carcinoma (OSCC) accounts for at least 40% of cancer cases. It is the most common cancer caused by the oral habits of smoking, reverse smoking, and chewing betel nut [1]. The percentage of cancer cases in Japan that are OSCC-related is between 1% and 2%, and it is steadily rising [2]. The quality of life for patients with OSCC is often negatively impacted by mastication, speech, and deglutition problems. Furthermore, there is still a poor prognosis for OSCC, throughout the previous 20 years, the overall 5-year survival rate has stayed at about 56% [3].

It was initially discovered that cortactin is one of the main substrates for src kinase [4]. It was named cortactin because it is confined to cortical actin structures [5]. Not much was known about its function, except that it linked to actin filaments, the presence of SH3 domain, and the phosphorylation of its C-terminus by src kinase. [5]. After this, it was discovered that the cortactin gene was identical to Ems1 [6], which is often overexpressed in head and neck malignancies because it is located in the 11q13 amplicon [7]. A poor prognosis has been linked to 11q13 amplification on multiple occasions, including advanced pathological stages, lymph node and distant metastases, and decreased survival rate [7–13]. This amplicon contains a large number of other genes. Nevertheless, cortactin is commonly found in cell motility structures such as invadopodia and lamellipodia [6,7] and the frequent overexpression of it in tumors with 11q13 amplification has sparked significant

interest in its role across various assays, including single-cell motility [16], wound healing [16,17], and transwell migration [14,15]. On the other hand, throughout many of the identical tests, siRNA targeting cortactin reduces cell motility [16,18–25]. Cortactin-deficient flies exhibit defective migration of border cells in egg chambers during Drosophila melanogaster oogenesis, revealing that cortactin influences cell migration both in vitro and in vivo [26]. In vivo, ECM breakdown is typically vital to cell migration through tissues. Since cortactin is required for invasion via matrix barriers such as transwell filters covered in gelatin or Matrigel, it seems to be a key player in this process [16,27–31]. Cortactin has a crucial role in subcellular invadopodia, actin-rich protrusions linked to the breakdown of extracellular matrix [32]. They distinguish themselves by colocalizing numerous proteins, including membrane trafficking proteins, proteases, focal adhesions, and lamellipodia [33]. Osteoclasts, macrophages, and other normal cells that have to penetrate tissue barriers or reorganize extracellular matrix (ECM) include podosome structures [34,35].

Cortactin is strongly localized to regions of focal ECM breakdown, it is commonly employed as an invadopodia marker. Cortactin is known to be an important protein that contributes to the formation of podosomes and invadopodia [36,37,38–42]. During invadopodia formation, actin polymerization was completed by the linking impact of Cortactin [43]. However, interestingly, studies found that cortactin promotes actin polymerization in podosomes but not in lamellipodia [44]. Cortactin downregulation also related with the podosome as well as invadopodia dynamics in cells that have undergone src transfection or overexpression [45, 46]. Cortactin has various bingding partners, and the cortactin phosphorylation can triggers various signaling pathways involving activation of those binding partners [46-48].

Many human malignancies, such as those of the head, neck, and esophagus, as well as colorectal, stomach, hepatocellular, breast, and ovarian cancers, overexpress cortactin [9,11,46–49]. Over-expression of CTTN was thought to be a poor prognostic predictor for head and neck cancer, which is consistent with its well-characterized, cell biology activities in cell motility and ECM disintegration [50]. Given its variety of binding partners, CTTN could serve many purposes. Using OSCC cell lines and tissues as a model, we assessed the expression of CTTN and its potential clinicopathological implications in OSCC patients. Given its variety of binding partners, CTTN can be assumed to perform various activities. Using OSCC cell lines and tissues as a model, we assessed the expression of CTTN and its potential clinicopathological implications in OSCC patients

2. Materials & Methods

2.1. Patients and samples

In this study, 198 patients with OSCC who underwent surgical treatment at Yonsei University Dental Hospital from 1995 to 2010 were selected. The median age of the cohort was 61 years (23-91 years). This cohort includes 72 (69.9%) male and 31(30.1%) female. Lesion sites included the 64 (35.9%) tongue, 9(4.5%) retromolar triangle, 12 (6.1%) retromolar trigone, 48 (24.2%) mandible, 34 (17.2%) maxilla, 29 (14.6%) buccal cheek, and 2 (1.0) lip. Patients with the T1-T2 stage accounted for 121 (61.1%) and patients with the T3-T4 stage accounted for 77 (38.9%). Lymph node involvement, vascular invasion, and perineural invasion were found in 51 (25.8%), 19 (9.6%), and 18 (9.1%) OSCC patients (Table 1). Each patient was followed for at least 5 years to assess long-term outcomes. The research protocol has received ethical approval from the Yonsei University Health System Dental Hospital Institutional Review Board (IRB No. 2-2011-0044). This approval ensures that the research is conducted by ethical guidelines, protecting the rights and well-being of all participants involved in the research.

Table 1. Clinicopathological characteristics of 103 patients with OSCC

Clinicopathological variables	No. of patients (%)
Total cases	103
Age, years	
Median age (range)	57(32-79)
≤60	57(55.3)
>60	46(44.7)
Gender	
Male	72(69.9)
Female	31(30.1)
Site	
Tongue	37(35.9)
Retromolar trigone	26(25.2)
Gingiva	40(38.8)
T stage	
T1-T2	43(41.7)
T3-T4	60(58.3)
N stage	
Negative	53(51.5)
Positive	50(48.5)
Vascular invasion	
Absent	57(55.3)
Present	46(44.7)
Perineural invasion	
Absent	78 (75.7)
Present	25(12.6)

2.2. Immunohistochemistry

OSCC paraffin-embedded tissues were cut into 4um and then performed immunohistochemical analysis. Sections were dewaxed with xylene, and then hydrated with a fractional alcohol solution. Antigen retrieval was performed using a Citrate buffer (Sigma-Aldrich, USA) via a pressure cooker, and endogenous peroxidase activity was blocked by 4% H_2O_2 (Junsei Chemical Co., Ltd, Japan). CTTN (Mouse monoclonal antibody against) primary antibody was purchased from Abcam (Cambridge, MA, USA) with working dilution 1:2000. Incubated at room temperature for 2 hours. The REAL EnVision Rabbit/rat HRP detection system from Dako (Carpinteria, CA, USA) was used for secondary antibody incubation and 3,3'-diaminobenzidine (DAB) color development. Counterstaining was performed using hematoxylin for 1 minute. We prepared phosphate-buffered saline (PBS) instead of a primary antibody as a negative control. The protein expression level was scored using the weighted tissue scoring method, and the total tissue score was calculated according to staining intensity and percentage of positive cells. For further analysis, patients were divided into two groups: a low expression group (total score 0-100) and a high expression group (total score 101-300) according to their total tissue scores.

2.3. Cell lines and cell culture

HSC-2 and CA9-22 OSCC cell lines were purchased from the Korean Cell Line Bank (Seoul, Korea). HyClone™ Minimum Essential Medium (MEM) with Earle's Balanced Salts (MEM/EBSS, HyClone Laboratories, Inc., Logan, UT, USA) culture medium was used. The supplement included 10% fetal bovine serum (FBS; Gibco BRL, Grand Island, NY, USA), 100 U/mL of penicillin, 100 mg/mL streptomycin, 0.4 g/mL hydrocortisone, 5 μg/mL transferrin, 5 μg/mL insulin, $1x10^{-10}$M cholera toxin, and $2x10^{-11}$M triiodothyronine. Cells were maintained in a 5% CO_2 cell culture incubator at 37°C.

2.4. Small interfering RNA (siRNA) transfection

CTTN expression was blocked by three independent pools of small interfering RNA (siRNA) oligonucleotides targeting CTTN. A specific number of cells were seeded in a 6-well plate. Before transfection, the cells starvated with serum-free and (-) P/S medium for 4h. CTTN targeting siRNAs as well as related scramble control (Invitrogen, Carlsbad, CA, USA) were transfected using Lipofectamine™ 2000 (Invitrogen, Carlsbad, CA, USA). Downregulation efficacy of *CTTN* mRNA expression was confirmed by real time PCR analysis.

2.5. Cell cycle and apoptosis assay by flow cytometry

Each group of OSCC cells were harvested and stained with propidium iodide using a Cell cycle Analysis kit (ab287852, Abcam, USA) according to manufacturer's protocol. Each group of stained cells determined by flow cytometry (LSR Fortessa, Becton Dickinson, USA). Apoptotic cells were determined using the FITC Annexin V Apoptosis detection kit I (BD Pharmingen™, Cat: 556547, USA). After stained with Annexin V-FITC and PI, population of stained cells were detected by flow cytometry.

2.6. Wound healing assay

Cells were seeded with culture media in 6-well culture plates. Incubate the cells at 37°C with 5% CO_2 until they form a confluent monolayer. Use a sterile pipette tip (200μL tip) to scrape a straight line through the cell layer. Gently wash the cells with sterile PBS (phosphate-buffered saline) to remove shed cells and debris. Replace the PBS with a fresh cell culture medium and immediately take images of the wound area using an inverted microscope. Return the cells to the incubator and allow them to migrate. At predetermined time points, take images of the same wound area. To assess wound healing, three points were randomly selected for marking. Using Image-J software (National Institutes of Health, Maryland, USA), the horizontal migration distance of cells from the starting point (0 hours) was measured and analyzed.

2.7. Transwell invasion assay

The cell invasion assay was performed using a cell culture insert (Falcon, NY, USA). Matrigel was diluted in serum-free medium added into the upper chambers and dried for 7h at RT for gel coating. Then, $1x10^5$ cells in medium were seeded into the coated upper chamber. For induction of cell invasion, 2% and 20% FBS was added in upper chamber and lower chamber, respectively. After 36 hours of cell seeding, invading cells were stained with 0.1% crystal violet and counted using microscope.

2.8. Total RNA extraction and reverse transcription PCR analysis

Total RNA was extracted with TRIzol reagent (Invitrogen, Carlsbad, CA, USA). After cDNA synthesis, 1X SYBR-Green Master Mix (Applied Biosystems) was used. The Mx3005P QPCR system (Agilent Technologies, Santa Clara, CA, USA) was supplemented with 10 pmol primer and 1.5μl cDNA for quantitative real-time PCR analysis under the following conditions: initial denaturation at 95°C for 10 min; Then 40 cycles of 95°C for 20 seconds, 50°C for 30 seconds, and 72°C for 45 seconds were performed. The oligonucleotide primers for PCR are listed in Table 2.

Table 2. Sequence of primers

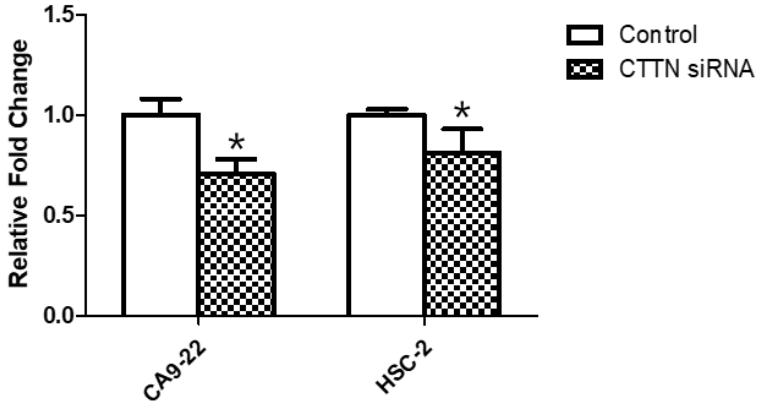
Primer	5′to3′
GAPDH	Forward:5’-GGCACAGTCAAGGCTGAGAATG-3’
	Reverse:5’-ATGGTGGTGAAGACGCCAGTA-3’
TGF-β	Forward:5’-GGGACTATCCACCTGCAAGA-3’
	Reverse:5’-CCTCCTTGGCGTAGTAGTCG-3’
IL-1α	Forward:5’-CAGAGGGCCTGTACCTCATC-3’
	Reverse:5’-GGAAGACCCCTCCCAGATAG-3’
IL-1β	Forward:5’-AGCCATGGCAGAAGTACCTG-3’
	Reverse:5’-CCTGGAAGGAGCACTTCATCT-3’
IL-17	Forward:5’-TGCCTTCAGCAGAGTGAAGA-3’
	Reverse:5’-GGTCTTGGTTCTCAGCTTGG-3’
PCNA	Forward:5’-ATTGGGATCATCTTGCTGGT-3
	Reverse:5’-GAGAGGCGCCTGATCTCTTC-3’

3. Results

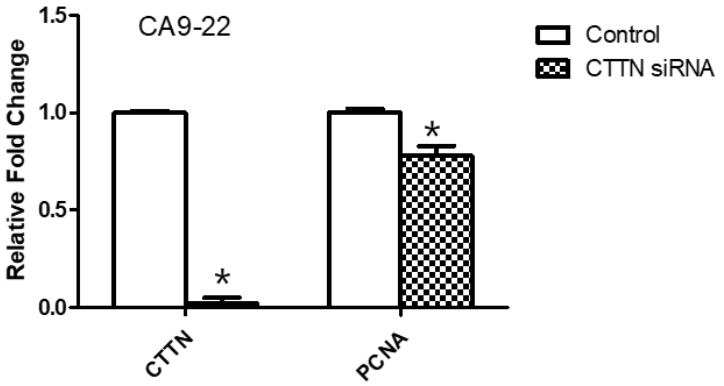
3.1. Influence of CTTN knockdown on proliferation of OSCC cell lines

In this study, viable cells were 0.71±0.07 and 0.82±0.13 fold decreased in cells with CTTN downregulation than the related control cells, both in CA9-22 and HSC-2 cells, respectively (i). Moreover, 0.78±0.05 and 0.81±0.06 fold decreased PCNA expression was also found in cells with CTTN downregulation than the related control cells both in CA9-22 and HSC-2 cells, respectively (ii).

(i)



(ii)



(iii)

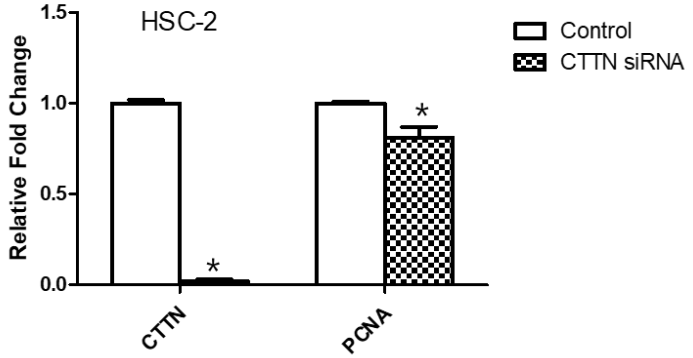


Figure 1. OSCC cell lines with CTTN Knockdown presented decreased proliferating ability. (i) Number of viable cells were significantly decreased in CTTN knockdown OSCC cell lines than the related control cells. (ii & iii) Both *CTTN* and *PCNA* mRNA expression was significantly decreased in CTTN knockdown OSCC cell lines than related control cells (**P*<0.05 vs. control).

3.2. Influence of CTTN knockdown on cell cycle analysis

In this study, S phase cell population decreased 0.47±0.09 and 0.82±0.06 fold in CTTN knockdown cells than the control cells in CA9-22 and HSC-2 cells, respectively (Figure 2).

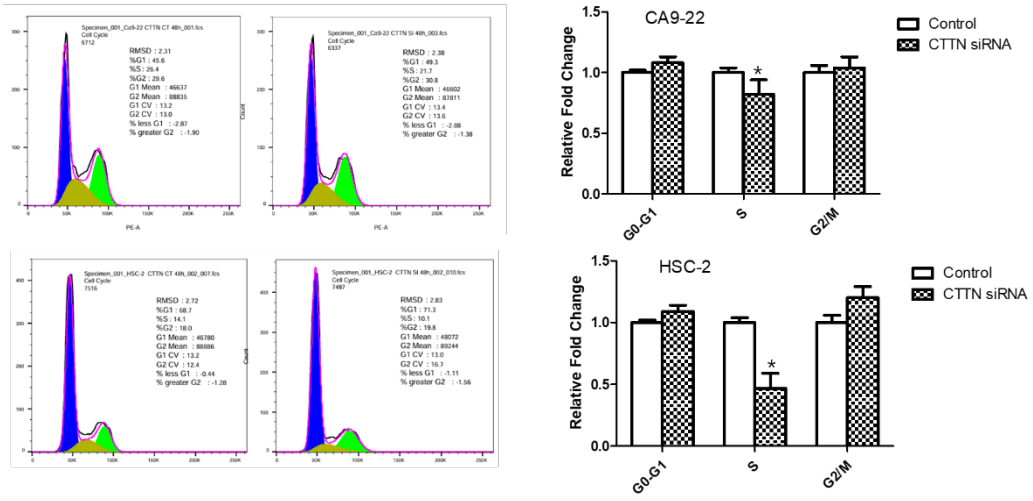


Figure 2. Influence of CTTN knockdown on cell cycle analysis. CTTN knockdown mediates significantly decreased S phase cell population in OSCC cell lines. Both CA9-22 and HSC-2 cell lines showed significant decrease in S phase population after CTTN siRNA transfection (*P<0.05 vs. control).

3.3. Influence of CTTN knockdown in apoptosis of OSCC cell lines

In flow cytometry analysis, both Ca9-22 (1.68±0.14 fold) and HSC-2 (1.90±0.16 fold) showed significantly increased late phase apoptosis after CTTN knockdown (Figure 3).

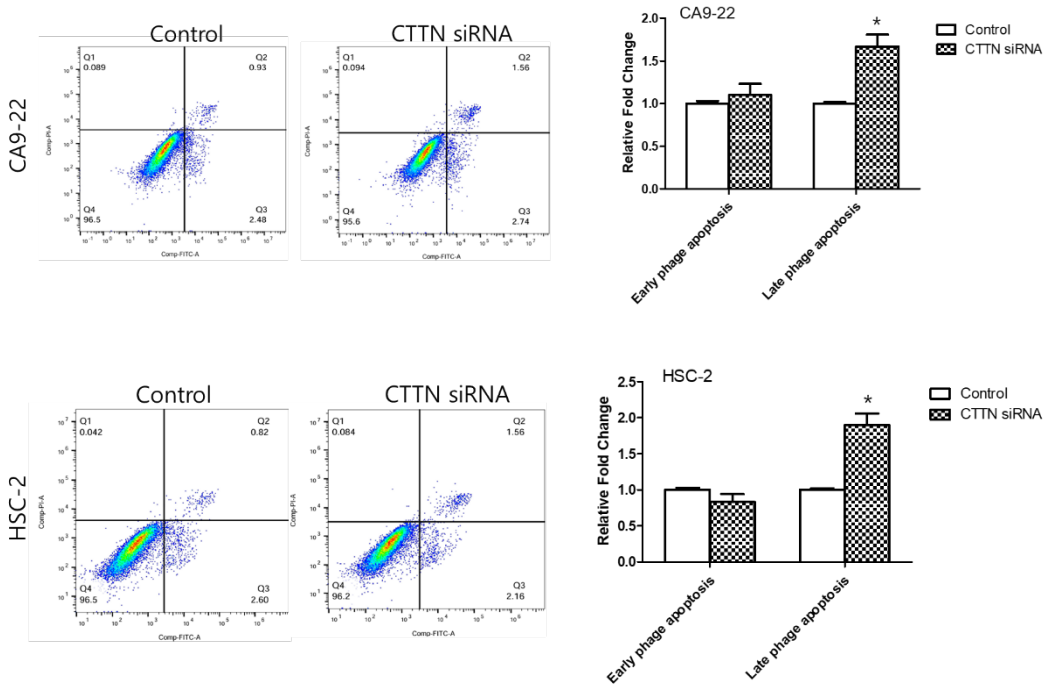


Figure 3. Influence of CTTN knockdown in apoptosis of OSCC cell lines. CTTN knockdown can increase late phase cell apoptosis in OSCC cell lines (*P<0.05 vs. control).

3.4. Effect of CTTN knockdown on motility of OSCC cells

In wound healing assay, wound closure rate was 0.61±0.03- and 0.45±0.12-fold decreased both in CTTN knockdown CA9-22 and HSC-2 cells than related control cells in this study (Figure 4).

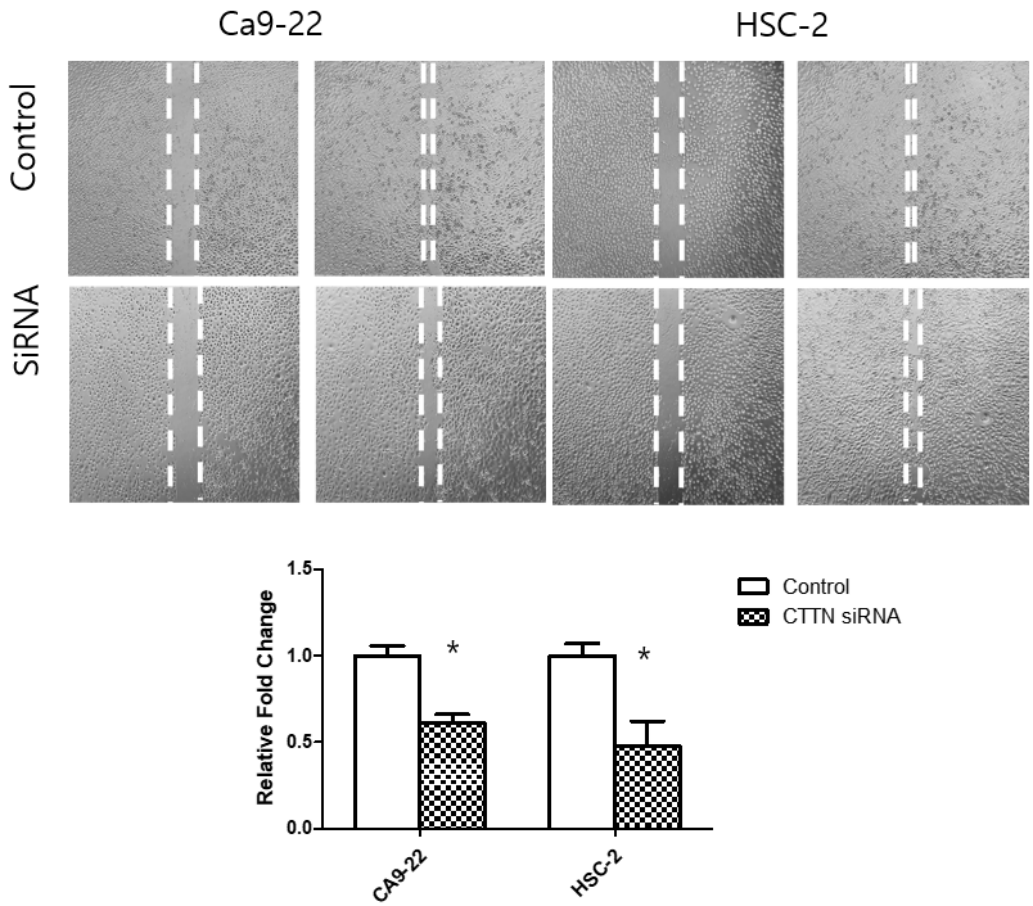


Figure 4. Effect of CTTN knockdown on motility of OSCC cells. CTTN knockdown can attenuate wound closure rate of OSCC cell lines (*P<0.05 vs. control).

3.5. Effect of CTTN knockdown on invasive capacity of OSCC cells

In this study, number of invading cells were 0.2±0.22- and 0.3±0.16-fold decreased after CTTN knockdown than the control cells, both in CA9-22 and HSC-2 cells (Figure 5).

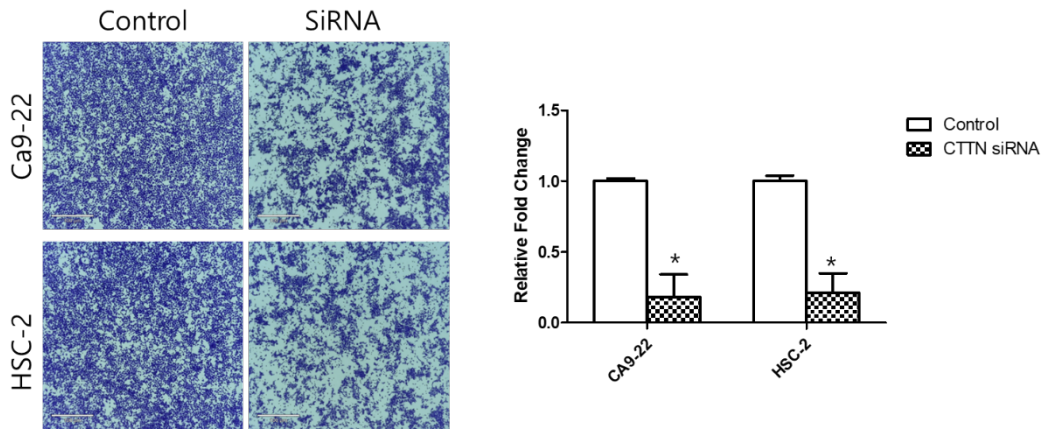
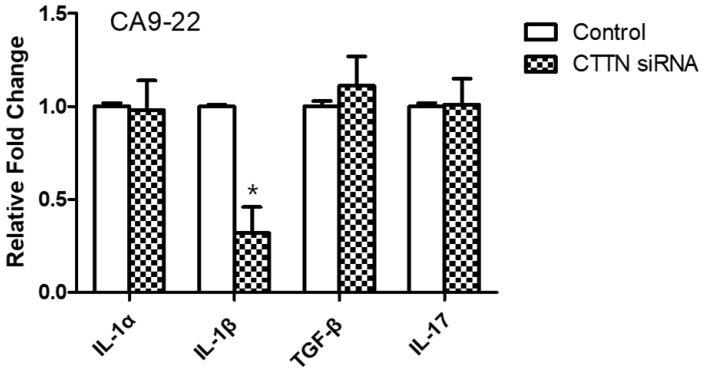


Figure 5. Effect of CTTN knockdown on invasive capacity of OSCC cells. CTTN knockdown can significantly decrease invasion ability of OSCC cell lines (*P<0.05 vs. control).

3.6. Effect of CTTN knockdown on cytokine expression of OSCC cells

To assess the influence of CTTN knockdown in cytokine expression of OSCC cells, cytokines of related to fibrosis in cancers were determined by real-time PCR analysis. Results showed that IL-1β expression was significantly decreased in CTTN knockdown cells compared to the related control cells in both CA9-22 and HSC-2 (Figure 6). There are no significant differences between each group of cells in IL-1α, TGF-β, and IL-17mRNA expression (Figure 6).

(i)



(ii)

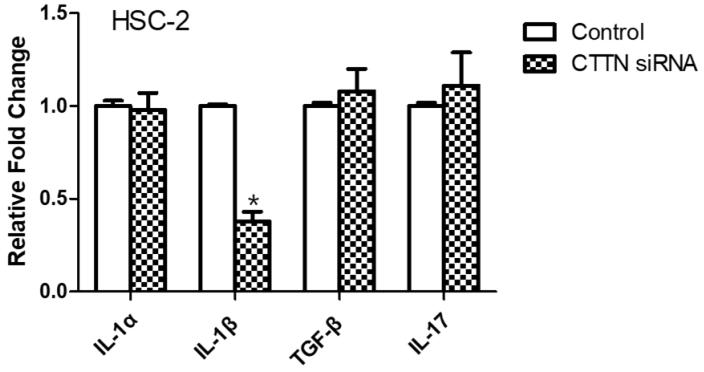


Figure 6. Effect of CTTN knockdown on cytokine expression of OSCC cells. CTTN knockdown significantly decreases IL-1β expression of OSCC cell lines (*P<0.05 vs. control).

3.7. Clinicopathological significance of CTTN expression in 103 OSCC patients

3.7.1. CTTN expression was significantly associated with desmoplastic reaction

CTTN expression was found in cytoplasm of tumor cells and stroma cells including vascular endothelial cells as well as cancer-associated fibroblasts in OSCC tissues. The representative expression patterns of CTTN in OSCC was shown in Figure 7. In our cohort, 64 (62.1%) patients showed desmoplastic reactions, and both tumoral and stromal CTTN expression was significantly related to desmoplastic reaction in OSCC patients (*P<0.05).

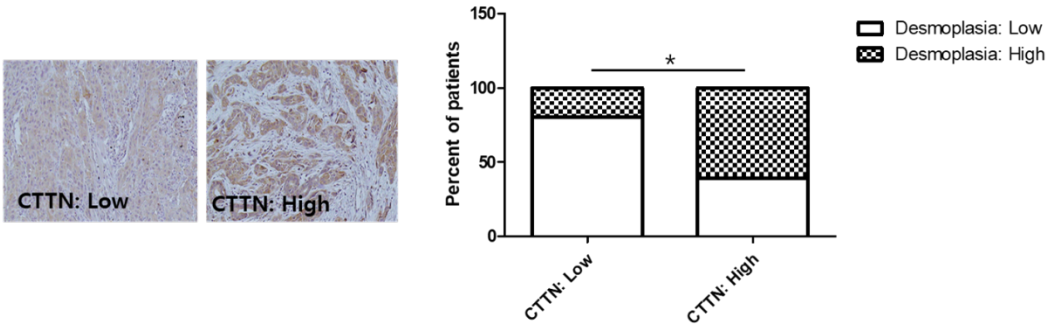


Figure 7. Representative expression patterns of CTTN in OSCC tissues. CTTN expression was significantly associated with desmoplastic reactions in OSCC patients.

3.7.2. Significant association was found between CTTN expression with IL-1β expression in OSCC patients.

IL-1β expression was found in both OSCC cells and stroma tissues. As a proinflammatory cytokines, IL-1βexpression was significantly associated with desmoplastic reaction as well as CTTN expression in our cohort (Figure 8).

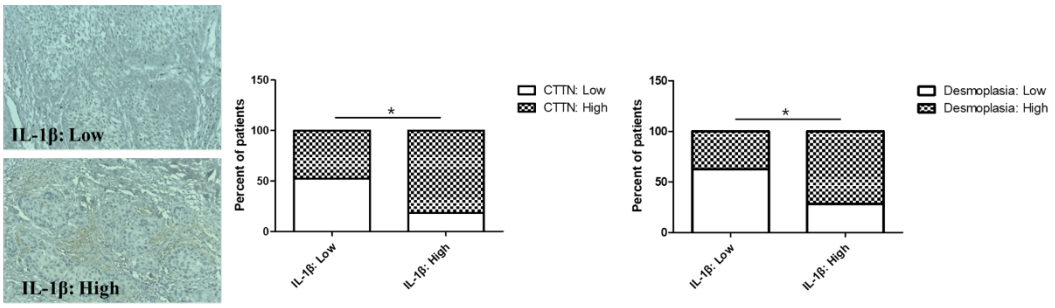


Figure 8. IL-1βexpression was significantly associated with desmoplastic reaction as well as CTTN expression in our cohort (*P<0.05).

3.7.3. Association was found between CTTN expression and clinicopathologic variables

A significant association was found between CTTN expression with LN metastasis in our cohort. There are no significant association was found between CTTN expression and T stage, vascular invasion, and perineural invasion in our cohort (Table 3). Furthermore, patients with high CTTN expression showed poor prognosis than patients with low CTTN expression (Figure 9).

Table 3. Relationship between tumoral CTTN expression and clinicopathological characteristics of 103 OSCC patients

Clinicopathologic variables	No. of cases (%)	CTTN expression		P
		Low	High	
T stage				
T1-T2	43(41.7)	33(76.7)	10(23.3)	n.s.
T3-T4	60(58.3)	33(55.0)	27(45.0)	
LN status				
Negative	53(51.5)	31(58.6)	22(41.4)	0.012
Positive	50(48.5)	14(27.8)	36(72.2)	
Vascular invasion				
Absent	57(55.3)	30(52.6)	27(47.4)	n.s.
Present	46(44.7)	29(63.0)	17(37.0)	
Perineural invasion				
Absent	71(68.9)	46(64.8)	25(35.2)	n.s.
Present	32(31.1)	17(53.2)	15(46.8)	

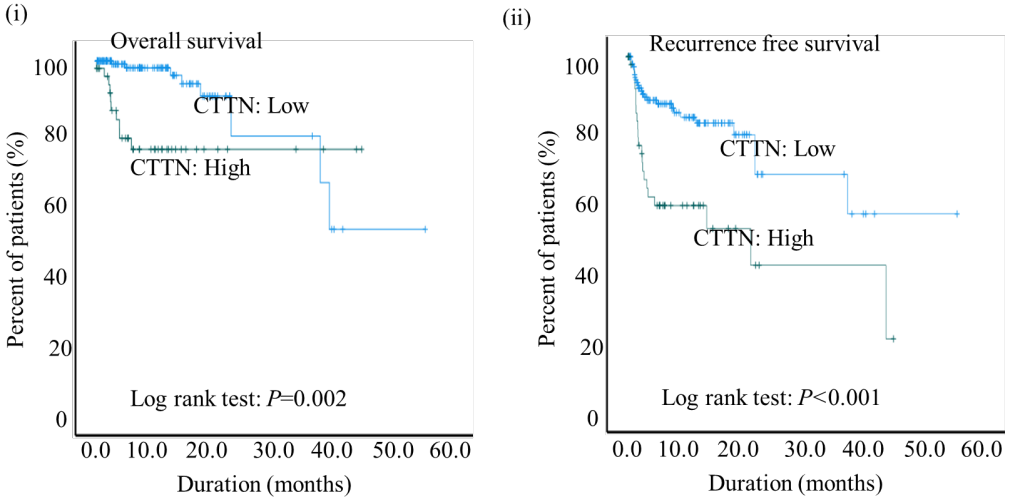


Figure 9. CTTN expression and corresponding prognosis. Overall- and recurrence-free survival period of patients with OSCC Patients with high CTTN expression showed poor overall- and recurrence-free survival than patients with low CTTN expression.

4. Discussion

OSCC is a type of cancerous tumor that often spreads to other regions by metastasizing from its initial invasion of the local bone and lymph nodes. Globally, there are currently about 500,000 new cases identified annually, and the 5-year survival rate is just about 50%. The response to varied therapy is significantly lower in advanced stage OSCC, and oral cancer continues to be a leading cause of mortality despite advancements in multimodal treatment and the fact that early-stage tumors respond effectively to combination therapy (Neville and Day, 2002). After surgical resection, speech and swallowing difficulties complicate treatment and can result in facial deformity or a poor prognosis overall. Cancer patients' morbidity and mortality are directly correlated with their invasiveness and potential for metastasis. In order to discover OSCC early and track the evolution of the cancer, it is necessary to comprehend the underlying mechanisms of targets for cancer therapy and biomarkers.

There is increasing proof relating the expression of disorder genes to cancer invasion and metastasis [20]. The carcinogenesis and metastasis of many malignant tumors, such as colon, breast, and esophageal squamous cell carcinoma, have been associated with aberrant activation of CTTN [16,21–23]. It hasn't been established yet how important CTTN is for prognosis in OSCC. Nevertheless, our findings demonstrated that OSCC tissues have elevated CTTN expression. Pathologic analysis of the tissues from OSCC patients revealed a strong relationship between CTTN expression and lymph node metastases and desmoplastic reaction, two well-known poor prognostic markers of OSCC. A Kaplan-Meier analysis revealed a lower overall survival time for patients with high CTTN expression. CTTN silencing in vitro suppressed the motility and invasive potential of OSCC cells. These findings suggest that CTTN might play a role as an oncogene in the advancement of OSCC.

The tumor microenvironment contains tumor cells that interact with surrounding cells via the lymphatic and circulatory systems, influencing cancer development and progression, and it has been widely associated with carcinogenesis [46]. Furthermore, by stimulating and facilitating uncontrolled cell proliferation, nonmalignant cells in the tumor microenvironment play crucial roles in all stages of carcinogenesis [47]. The cancer literature has extensively investigated

the tumor microenvironment, emphasizing its function in the development and progression of tumors. Numerous elements of the tumor microenvironment have been found to affect the behavior and progress of malignancy in previous research [45–51]. The tumor microenvironment includes malignant cells as well as tumor vasculature, lymphocytes, adipocytes, dendritic cells, fibroblasts, and cancer-associated fibroblasts [45]. The diverse immune capacities of each of these cell types affect whether the tumor can survive and impact surrounding cells. This study revealed a considerable increase in CTTN expression in the stroma cells of OSCC tissues, but no significant prognostic effect was observed in our experimental cohort. The clinicopathologic relevance of CTTN expression in various cell types of OSCC stroma tissues may need further investigation.

The term "desmoplasia" describes the development of stroma, or dense connective tissue [23]. Desmoplasia has been studied as a prospective useful predictive factor for survival because it is a common indicator of wound healing and cancer progression. In this study, we found a positive correlation between desmoplastic reaction and CTTN expression in patients with OSCC. Furthermore, CTTN knockdown resulted in a significant decrease in cytokines associated with desmoplasia reactions, such as IL-1ß expression.

5. Conclusion

We concluded that CTTN overexpression can increase the oncogenic activity of OSCC cells via promotes the ability of proliferation, migration, and invasion. Moreover, CTTN may also can increase desmoplastic reactions via promotes cytokine expression of cancer cells that can trigger fibrosis in OSCC patients. Further studies were needed for investigate the underlying molecular mechanism of CTTN in OSCC progression.

References

[1] Bach D, Naon D, Pich S, Soriano FX, Vega N, Rieusset J, et al.: Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects o f type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. *Diabetes* 54(9): 2685-2693, 2005.

[2] Bagchi A, Mills AA: The quest for the 1p36 tumor suppressor. *Cancer Res* 68(8): 255 1-2556, 2008.

[3] Bereiter-Hahn J, Voth M: Dynamics of mitochondria in living cells: shape changes, disl ocations, fusion, and fission of mitochondria. *Microsc Res Tech* 27(3): 198-219, 1994.

[4] Campello S, Scorrano L: Mitochondrial shape changes: orchestrating cell pathophysiolog y. *EMBO Rep* 11(9): 678-684, 2010.

[5] de Brito OM, Scorrano L: Mitofusin 2: a mitochondria-shaping protein with signaling r oles beyond fusion. *Antioxid Redox Signal* 10(3): 621-633, 2008.

[6] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127(12): 2893-2917, 201 0.

[7] Hernandez-Alvarez MI, Thabit H, Burns N, Shah S, Brema I, Hatunic M, et al.: Subje cts with early-onset type 2 diabetes show defective activation of the skeletal muscle P GC-1{alpha}/Mitofusin-2 regulatory pathway in response to physical activity. *Diabetes Care* 33(3): 645-651, 2010.

[8] Jin B, Fu G, Pan H, Cheng X, Zhou L, Lv J, et al.: Anti-tumour efficacy of mitofusin -2 in urinary bladder carcinoma. *Med Oncol* 28 Suppl 1: S373-380, 2011.

[9] Kademani D, Bell RB, Bagheri S, Holmgren E, Dierks E, Potter B, et al.: Prognostic f actors in intraoral squamous cell carcinoma: the influence of histologic grade. *J Oral Maxillofac Surg* 63(11): 1599-1605, 2005.

[10] Kluge MA, Fetterman JL, Vita JA: Mitochondria and endothelial function. *Circ Res* 11 2(8): 1171-1188, 2013.

[11] Lee EJ, Kim J, Lee SA, Kim EJ, Chun YC, Ryu MH, et al.: Characterization of new ly established oral cancer cell lines derived from six squamous cell carcinoma and two mucoepidermoid carcinoma cells. *Exp Mol Med* 37(5): 379-390, 2005.

[12] Lou Y, Li R, Liu J, Zhang Y, Zhang X, Jin B, et al.: Mitofusin-2 over-expresses and leads to dysregulation of cell cycle and cell invasion in lung adenocarcinoma. *Med Oncol* 32(4): 132, 2015.

[13] Lugus JJ, Ngoh GA, Bachschmid MM, Walsh K: Mitofusins are required for angiogenic function and modulate different signaling pathways in cultured endothelial cells. *J Mol Cell Cardiol* 51(6): 885-893, 2011.

[14] Matsuda T, Marugame T, Kamo K, Katanoda K, Ajiki W, Sobue T, et al.: Cancer incidence and incidence rates in Japan in 2006: based on data from 15 population-based cancer registries in the monitoring of cancer incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 42(2): 139-147, 2012.

[15] Neville BW, Day TA: Oral cancer and precancerous lesions. *CA Cancer J Clin* 52(4): 195-215, 2002.

[16] Petruzzelli GJ: The biology of tumor invasion, angiogenesis and lymph node metastasis. *ORL J Otorhinolaryngol Relat Spec* 62(4): 178-185, 2000.

[17] Petruzzelli GJ: The biology of distant metastases in head and neck cancer. *ORL J Otorhinolaryngol Relat Spec* 63(4): 192-201, 2001.

[18] Ranieri M, Brajkovic S, Riboldi G, Ronchi D, Rizzo F, Bresolin N, et al.: Mitochondrial fusion proteins and human diseases. *Neurol Res Int* 2013: 293893, 2013.

[19] Shen T, Zheng M, Cao C, Chen C, Tang J, Zhang W, et al.: Mitofusin-2 is a major determinant of oxidative stress-mediated heart muscle cell apoptosis. *J Biol Chem* 282(32): 23354-23361, 2007.

[20] Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC: Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. *Mol Biol Cell* 20(15): 3525-3532, 2009.

[21] Wang W, Xie Q, Zhou X, Yao J, Zhu X, Huang P, et al.: Mitofusin-2 triggers mitochondria Ca2+ influx from the endoplasmic reticulum to induce apoptosis in hepatocellular carcinoma cells. *Cancer Lett* 358(1): 47-58, 2015.

[22] Wang W, Zhu F, Wang S, Wei J, Jia C, Zhang Y, et al.: HSG provides antitumor efficacy on hepatocellular carcinoma both in vitro and in vivo. *Oncol Rep* 24(1): 183-188, 2010.

[23] Westermann B: Molecular machinery of mitochondrial fusion and fission. *J Biol Chem*

283(20): 13501-13505, 2008.

[24] White JP, Baltgalvis KA, Puppa MJ, Sato S, Baynes JW, Carson JA: Muscle oxidative capacity during IL-6-dependent cancer cachexia. *Am J Physiol Regul Integr Comp Physiol* 300(2): R201-211, 2011.

[25] Witton CJ, Hawe SJ, Cooke TG, Bartlett JM: Cyclooxygenase 2 (COX2) expression is associated with poor outcome in ER-negative, but not ER-positive, breast cancer. *Histopathology* 45(1): 47-54, 2004.

[26] Youle RJ, van der Bliek AM: Mitochondrial fission, fusion, and stress. *Science* 337(6098): 1062-1065, 2012.

[27] Zhang GE, Jin HL, Lin XK, Chen C, Liu XS, Zhang Q, et al.: Anti-tumor effects of Mfn2 in gastric cancer. *Int J Mol Sci* 14(7): 13005-13021, 2013.

[28] Zhang X, Zheng Z, Yingji S, Kim H, Jin R, Renshu L, et al.: Downregulation of glutathione peroxidase 3 is associated with lymph node metastasis and prognosis in cervical cancer. *Oncol Rep* 31(6): 2587-2592, 2014.

[29] Bach D, Naon D, Pich S, Soriano FX, Vega N, Rieusset J, et al.: Expression of Mfn2, the Charcot-Marie-Tooth neuropathy type 2A gene, in human skeletal muscle: effects of type 2 diabetes, obesity, weight loss, and the regulatory role of tumor necrosis factor alpha and interleukin-6. *Diabetes* 54(9): 2685-2693, 2005.

[30] Bagchi A, Mills AA: The quest for the 1p36 tumor suppressor. *Cancer Res* 68(8): 251-2556, 2008.

[31] Bereiter-Hahn J, Voth M: Dynamics of mitochondria in living cells: shape changes, dislocations, fusion, and fission of mitochondria. *Microsc Res Tech* 27(3): 198-219, 1994.

[32] Campello S, Scorrano L: Mitochondrial shape changes: orchestrating cell pathophysiology. *EMBO Rep* 11(9): 678-684, 2010.

[33] de Brito OM, Scorrano L: Mitofusin 2: a mitochondria-shaping protein with signaling roles beyond fusion. *Antioxid Redox Signal* 10(3): 621-633, 2008.

[34] Ferlay J, Shin HR, Bray F, Forman D, Mathers C, Parkin DM: Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 127(12): 2893-2917, 2010.

[35] Hernandez-Alvarez MI, Thabit H, Burns N, Shah S, Brema I, Hatunic M, et al.: Subjects with early-onset type 2 diabetes show defective activation of the skeletal muscle P

GC-1{alpha}/Mitofusin-2 regulatory pathway in response to physical activity. *Diabetes Care* 33(3): 645-651, 2010.

[36] Jin B, Fu G, Pan H, Cheng X, Zhou L, Lv J, et al.: Anti-tumour efficacy of mitofusin-2 in urinary bladder carcinoma. *Med Oncol* 28 Suppl 1: S373-380, 2011.

[37] Kademani D, Bell RB, Bagheri S, Holmgren E, Dierks E, Potter B, et al.: Prognostic factors in intraoral squamous cell carcinoma: the influence of histologic grade. *J Oral Maxillofac Surg* 63(11): 1599-1605, 2005.

[38] Kluge MA, Fetterman JL, Vita JA: Mitochondria and endothelial function. *Circ Res* 112(8): 1171-1188, 2013.

[39] Lee EJ, Kim J, Lee SA, Kim EJ, Chun YC, Ryu MH, et al.: Characterization of newly established oral cancer cell lines derived from six squamous cell carcinoma and two mucoepidermoid carcinoma cells. *Exp Mol Med* 37(5): 379-390, 2005.

[40] Lou Y, Li R, Liu J, Zhang Y, Zhang X, Jin B, et al.: Mitofusin-2 over-expresses and leads to dysregulation of cell cycle and cell invasion in lung adenocarcinoma. *Med Oncol* 32(4): 132, 2015.

[41] Lugus JJ, Ngoh GA, Bachschmid MM, Walsh K: Mitofusins are required for angiogenic function and modulate different signaling pathways in cultured endothelial cells. *J Mol Cell Cardiol* 51(6): 885-893, 2011.

[42] Matsuda T, Marugame T, Kamo K, Katanoda K, Ajiki W, Sobue T, et al.: Cancer incidence and incidence rates in Japan in 2006: based on data from 15 population-based cancer registries in the monitoring of cancer incidence in Japan (MCIJ) project. *Jpn J Clin Oncol* 42(2): 139-147, 2012.

[43] Neville BW, Day TA: Oral cancer and precancerous lesions. *CA Cancer J Clin* 52(4): 195-215, 2002.

[44] Petruzzelli GJ: The biology of tumor invasion, angiogenesis and lymph node metastasis. *ORL J Otorhinolaryngol Relat Spec* 62(4): 178-185, 2000.

[45] Petruzzelli GJ: The biology of distant metastases in head and neck cancer. *ORL J Otorhinolaryngol Relat Spec* 63(4): 192-201, 2001.

[46] Ranieri M, Brajkovic S, Riboldi G, Ronchi D, Rizzo F, Bresolin N, et al.: Mitochondrial fusion proteins and human diseases. *Neurol Res Int* 2013: 293893, 2013.

[47] Shen T, Zheng M, Cao C, Chen C, Tang J, Zhang W, et al.: Mitofusin-2 is a major

determinant of oxidative stress-mediated heart muscle cell apoptosis. *J Biol Chem* 282(32): 23354-23361, 2007.

[48] Song Z, Ghochani M, McCaffery JM, Frey TG, Chan DC: Mitofusins and OPA1 mediate sequential steps in mitochondrial membrane fusion. *Mol Biol Cell* 20(15): 3525-3532, 2009.

[49] Wang W, Xie Q, Zhou X, Yao J, Zhu X, Huang P, et al.: Mitofusin-2 triggers mitochondria Ca2+ influx from the endoplasmic reticulum to induce apoptosis in hepatocellular carcinoma cells. *Cancer Lett* 358(1): 47-58, 2015.

[50] Wang W, Zhu F, Wang S, Wei J, Jia C, Zhang Y, et al.: HSG provides antitumor efficacy on hepatocellular carcinoma both in vitro and in vivo. *Oncol Rep* 24(1): 183-188, 2010.

[51] Westermann B: Molecular machinery of mitochondrial fusion and fission. *J Biol Chem* 283(20): 13501-13505, 2008.

ABSTRACT (in Korean)

구강 편평세포암의 발병 기전에서 Cortactin 발현의 의미

<지도교수 남 웅>

김 민 지

구강 편평세포암(oral squamous cell carcinoma, OSCC)의 조기 진단과 치료는 구강악안면외과학 분야에서 공통적인 도전 과제이다. 암 조직에서 invadosome의 주요 구조 단백질인 Cortactin(CTTN)은 암세포의 생물학적 역할에 다양한 변화를 일으킬 수 있지만, 관련된 분자 메커니즘은 뚜렷하게 알려진 바가 없다.

본 연구에서는 인비트로(in vitro) 검사로서 CTTN 발현이 OSCC 세포의 생물학적 행동에 미치는 영향을 평가하였다. 또한 OSCC 코호트(cohort)에서 CTTN 발현의 임상병리학적 의의를 연구하였다.

CTTN 녹다운(knockdown) OSCC 세포는 대조군에 비해 세포의 증식(proliferation), 이동(motility) 및 침윤(invasion) 능력이 감소했으며, 세포 사멸(apoptosis)을 증가시킴을 확인하였다. 또한 CTTN knockdown OSCC 세포에서는 대조군에 비해 IL-1β 발현이 유의미하게 감소함을 확인하였고, 이와 함께 OSCC 코호트에서 IL-1β와 CTTN 발현 사이의 유의미한 연관성을 확인하였다. OSCC 코호트에서 CTTN 과발현은 림프절 전이, 결합조직형성(desmoplastic reaction), 그리고 불량한 예후(prognosis)와 양의 상관관계를 보였다.

이를 종합적으로 고려했을 때, CTTN은 OSCC에서 발암 활성(oncogenic activity)을 가지며, 이는 CTTN이 OSCC 환자의 새로운 진단 및 예후를 가늠하는 바이오마커(biomarker)로서의 가능성을 시사한다.

핵심되는 말: CTTN, 림프절 전이, 결합조직형성, 분자 바이오마커, OSCC