

Skp2 Regulates the Expression of MMP-2 and MMP-9, and Enhances the Invasion Potential of Oral Squamous Cell Carcinoma

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Abstract Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck regions and accounts for more than 90 % of cancers in the oral cavity. S-phase kinase-associated protein-2 (Skp2) is a member of the F-box protein family and the substrate recognition subunit of the Skp1-Cullin-F box protein E3 ubiquitin ligase complex. Skp2 is oncogenic and overexpressed in human cancers. The aims of the present study were to determine the clinicopathological significance of Skp2 in OSCC and clarify its function in OSCC cell lines in vitro. Multiple methods including immunohistochemical staining, RT-PCR, western blotting, migration and invasion assays, and siRNA transfection were employed in order to investigate the clinicopathological significance and molecular function of Skp2 in OSCC. The overexpression of Skp2 was more frequent in OSCC than in the normal oral epithelium. It was also more frequently detected in cancers with higher grades according to the T classification, N classification, and pattern of invasion. The high-Skp2 expression group had a significantly poorer prognosis, at 30.1 %, than that of the low-expression group, at 63.0 %. The

downregulation of Skp2 decreased migration and invasion potentials in HSC3 cells. Moreover, the suppression of Skp2 reduced the enzyme activities of MMP-2 and MMP-9 via Sp1. Skp2 may be a prognostic factor in OSCC patients, and may also play crucial roles in the migration and invasion potentials of OSCC cells.

Keywords Skp2 · invasion · metastasis · migration · oral squamous cell carcinoma

Abbreviations

OSCC	Oral squamous cell carcinoma
Skp2	S-phase kinase-associated protein-2
MMP-2	Matrix metalloproteinase-2
MMP-9	Matrix metalloproteinase-9
TIMP-1	Tissue inhibitor of matrix metalloproteinase-1

Introduction

Oral squamous cell carcinoma (OSCC) is the most common malignant tumor of the head and neck regions and accounts for more than 90 % of cancers in the oral cavity [1]. The primary therapeutic modality for OSCC is surgery. Although recent advances in surgical techniques and anticancer agents have improved tumor regression and survival in patients with OSCC, wide surgical resection of OSCC inevitably leads to various oral dysfunctions. Therefore, new treatment strategies are urgently needed.

The presence of neck lymph node metastasis has been strongly associated with a poor prognosis in patients with squamous cell carcinoma of the head and neck [2–4]. Previous studies reported that a relationship existed between alterations in the expression of adhesion-related molecules

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and a poor prognosis in OSCC patients [5–8]. Furthermore, several tissue and biological markers have been identified as possible indicators of the aggressiveness and metastatic capability of tumors [9]. Metastasis to the regional lymph nodes and distant organs is an obstacle to the survival of OSCC patients. The initial steps in the sequential process of metastasis were previously found to be similar to epithelial-mesenchymal transition (EMT), in which cells lose epithelial characteristics including cell adhesion and gain mesenchymal features including cell motility during embryogenesis and wound healing [10, 12]. The acquisition of EMT, accompanied by the functional loss of E-cadherin, which maintains intercellular adhesion, was previously reported to stimulate the dissemination of single tumor cells from primary sites through cell-to-cell contact, thereby endowing cells with metastatic abilities [10–12].

S-phase kinase-associated protein-2 (Skp2) is a member of the F-box protein family and the substrate recognition subunit of the Skp1-Cullin-F box protein E3 ubiquitin ligase complex [13]. Skp2 is known to mediate the ubiquitination and degradation of the cyclin-dependent kinase inhibitor p27 [14, 15]. A reduction in p27 was previously reported in various human cancers and correlated with a poor prognosis [16]. Since Skp2 plays an important role in inducing the degradation of p27, it has been reported to be oncogenic and overexpressed in human cancers [17–24]. Skp2 was recently found to be crucially involved in the positive regulation of cancer stem cell populations and self-renewal ability [25]. Moreover, Yang et al. [26] reported that the acquisition of EMT was associated with the expression of Skp2 in paclitaxel-resistant breast cancer cells. Although a relationship has already been reported between the expression of Skp2 and a poor prognosis in various cancers [18, 21, 22, 24], those between the expression of Skp2 and clinicopathological features in OSCC have not yet been investigated.

Therefore, the aims of the present study were to determine the clinicopathological significance of Skp2 in OSCC and clarify its function in OSCC cell lines *in vitro*. We performed an immunohistochemical analysis to determine the relationships between the expression of Skp2 and clinicopathological features in clinical OSCC samples. We also examined the effects of Skp2 expression on the migration and invasion potential of OSCC cell lines.

Materials and Methods

Patients

The study protocol was approved by the Ethics Committee of Nagasaki University Graduate School of Biomedical Sciences (No.15010873). Paraffin-embedded sections were obtained from the biopsy specimens of 70 patients with OSCC who

underwent radical surgery in our department. Tumor stages were classified according to the TNM classification of the International Union against Cancer, histological differentiation was defined according to the WHO classification, and invasion patterns were determined according to Bryne's classification [27]. As controls, samples of a normal oral epithelium were obtained after informed consent from ten patients undergoing the routine surgical removal of their third molars.

Cell Lines

We examined the expression of Skp2 in seven OSCC cell lines (Ca9–22, SAS, SCC25, OSC20, HSC2, HSC3, and HSC4), which were obtained from the Human Science Research Resource Bank (Osaka, Japan). Of these, the expression of Skp2 was the strongest in the HSC3 cell line (data not shown). Therefore, we used the HSC3 cell line in our experiments. These cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Wako Pure Chemical Industries, Ltd., Osaka, Japan) supplemented with 10 % fetal bovine serum (FBS) (Sigma Chemical Co., St. Louis, MO, USA) under the conditions of 5 % CO₂ in air.

Immunohistochemical Staining and Evaluations

Serial 4- μ m-thick specimens were taken from tissue blocks. Sections were deparaffinized in xylene, soaked in target retrieval solution buffer (Dako, Glostrup, Denmark), and placed in an autoclave at 121 °C for 5 min for antigen retrieval. Endogenous peroxidase was blocked by incubating sections with 0.3 % H₂O₂ in methanol for 30 min. Immunohistochemical staining was performed using the Envision system (Envision+, Dako, Carpinteria, CA). The primary antibody used was directed against Skp2 (H-435, Santa Cruz Biotechnology, Inc., Texas, USA). Sections were incubated with the primary antibody overnight at 4 °C. Reaction products were visualized by immersing the sections in diaminobenzidine (DAB) solution, and the samples were counterstained with Meyer's hematoxylin and then mounted. Negative controls were prepared by replacing the primary antibody with phosphate-buffered saline. The expression of Skp2 was defined as the presence of specific staining mainly in the cytoplasm and nuclei of tumor cells. The immunoreactivity of Skp2 was scored based on the staining intensity and immunoreactive cell percentage as follows [28–30]: staining index 0 = tissue with no staining; 1 = tissue with faint or moderate staining in ≤ 25 % of tumor cells; 2 = tissue with moderate or strong staining in 25 % to 50 % of tumor cells; 3 = tissue with strong staining in ≥ 50 % of tumor cells. The overexpression of Skp2 was defined as a staining index ≥ 2 .

RNA Isolation and Semiquantitative Reverse Transcription-Polymerase Chain Reaction (RT-PCR)

Total RNA was isolated with TRIzol Reagent (Invitrogen, Carlsbad, CA, USA) and first-strand cDNA was synthesized from 1 μ g of total RNA using an Oligo d (T) primer (Invitrogen) and ReverTra Ace (Toyobo, Osaka, Japan). In the PCR analysis, cDNA was amplified by *Taq* DNA polymerase (Takara, Otsu, Japan). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as the endogenous expression standard. Each PCR program involved a 3-min initial denaturation step at 94 °C, followed by 25 cycles (for Skp2 and Sp1), 28 cycles (for MMP-2 and MMP-9), or 19 cycles (for GAPDH) at 94 °C for 30 s, 55 °C for 30 s, and 72 °C for 1 min, on a PCR Thermal Cycler MP (Takara). Primer sequences were as follows: 5'-TGAGCTGAACCTCTCCTGGT-3' for Skp2 (F); 5'-CTGGCACGATTCCAAAAACT-3' for Skp2 (R); 5'-CACTTTCCTGGGCAACAAAT-3' for MMP-2 (F); 5'-TGATGTCATCTGGGACAGA-3' for MMP-2 (R); 5'-TTCATCTTCCAAGGCCAATC-3' for MMP-9 (F); 5'-CAGAAGCCCCACTTCTTGTC-3' for MMP-9 (R); 5'-GGAGAGCAAACCAGCAGAC-3' for Sp1 (F); 5'-AAGGTGATTGTTTGGGCTTG-3' for Sp1 (R); 5'-ATGTCGTGGAGTCTACTGGC-3' for GAPDH (F); and 5'-TGACCTGCCACAGCCTTG-3' for GAPDH (R). The amplified products were separated by electrophoresis on ethidium bromide-stained 2 % agarose gels. Band intensity was quantified by Image J software.

Invasion Assay

A BioCoat Matrigel invasion chamber (Becton Dickinson, Bedford, MA) was used for the invasion assay. This contained an internal chamber with an 8- μ m porous membrane bottom that was coated with Matrigel. Six-well cell culture inserts and a 6-well multiwell companion plate were used for the experiment. The membranes were rehydrated with warm serum-free medium for 2 h. The internal chamber was filled with 1.25×10^5 cells in medium containing 10 % FBS as a chemoattractant. Cells were incubated for 72 h at 37 °C in a 5 % CO₂ atmosphere. After being incubated, non-invading cells were removed from the top of the wells with a cotton swab, and cells that transferred to the inverse surface of the membrane were subjected to Diff-Quick staining (Sysmex International Reagents Co., Ltd. Kobe, Japan). Cells were counted under a microscope at 100 \times magnification. As a control, cells that passed through a control chamber without Matrigel were counted. All experiments were performed in triplicate, and cell numbers were counted in at least 4 fields/well. The ratio of the cell count that passed through the Matrigel chamber to the control cell count was defined as the invasion index, and expressed as a percentage.

Wound Healing Assay

Cell migration was evaluated by a scratched wound-healing assay on plastic plate wells. In brief, cells were grown to confluence and then wounded using a pipette tip. Three wounds were made for each sample, and all were photographed at 0 h and subsequent time points. Cell migration was evaluated by measuring the width of the wound at identical positions.

RNA Interference (RNAi)

Skp2 siRNA sequences were 5'-GUACAGCACAUGG ACCUAUTT-3' and 5'-AUAGGUCCAUGUGCUG UACTT-3'. Scrambled control siRNA sequences were 5'-CGUAUGCGCGUACUCUAAUTT-3' and 5'-TTGCAUACGCGCAUGAGAUUA-3'. All sequences were submitted to the National Institutes of Health Blast program to ensure gene specificity.

All siRNAs were purchased from Takara Bio Inc. (Otsu, Japan). Cells were transfected with double-stranded RNA using TransIT-siQUEST[®] transfection reagent (Mirus, Madison, WI, USA) according to the manufacturer's protocol. The HSC3 cell line was used for this experiment. Briefly, 1.0×10^5 HSC3 cells were plated on each well of a six-well plate and allowed to grow for 24 h until they reached 50 % confluence. Cells were then transfected with siRNA at a concentration of 200 nM using the transfection reagent in serum-free medium. Following a 24-h incubation, the medium was replaced with serum-enriched medium and cells were cultured for an additional 24 h.

Western Blot Analysis

Cells were harvested by trypsinization, washed, and precipitated by centrifugation. The Mammalian Cell Extraction Kit (BioVision Research Products, Mountain View, CA) was used to extract proteins. All subsequent manipulations were performed on ice. Cells were incubated in Extraction Buffer Mix. The lysed cells were centrifuged at 15,000 rpm for 3 min, and the resultant supernatant was used as the cytoplasmic fraction. The protein concentration of each sample was measured with the micro-BCA protein assay reagent (Pierce Chemical Co., Rockford, USA). Samples were denatured in SDS sample buffer and loaded onto 12.5 % polyacrylamide gels. After electrophoresis, the proteins were transferred onto polyvinylidene difluoride membranes and immunoblotted with an anti-Skp2 antibody (H-435, Santa Cruz Biotechnology, Inc., Texas, USA) or anti- β -actin antibody (Cell Signaling, MA, USA). An incubation was performed with a horseradish peroxidase-conjugated secondary antibody (ECL antmouse IgG, Amersham Biosciences, Piscataway,

NJ; 0.01 $\mu\text{g/ml}$), and signals were visualized with an ECL Kit (Amersham Pharmacia Biotech, Buckinghamshire, UK).

Gelatin Zymography

The proteolytic activity of MMPs was assessed by SDS-PAGE using a Novex zymogram gel (Invitrogen) containing 0.1 % (m/v) gelatin. Cells were transfected with Skp2 siRNA, mock, or scrambled control siRNA, followed by an incubation for 24 h, and were rinsed with PBS and then incubated in serum-free medium for 48 h. Conditioned media were subjected to zymography. Following electrophoresis at 4 °C, the gels were incubated in Novex zymogram renaturation buffer (Invitrogen) at room temperature for 30 min and agitated with Novex zymogram development buffer (Invitrogen) at 37 °C overnight. Clear bands indicative of gelatinolytic activity were visualized by staining the gels with Coomassie blue.

Statistical Analysis

Statistical analyses were performed using StatMate® (ATMS Co., Tokyo, Japan). The relationships between the expression of Skp2 and clinicopathological features were assessed by Fischer's exact test. Continuous data are given as the means \pm standard deviation. Data sets were examined by a one-way analysis of variance (ANOVA) followed by Scheffé's post-hoc test. A survival analysis was carried out with Kaplan-Meier curves and the related Log-rank tests. *P* values less than 0.05 were considered significant.

Results

Relationships between Skp2 Expression and Clinicopathological Features

Immunohistochemistry with an anti-Skp2 polyclonal antibody was performed on samples obtained from 70 patients with OSCC. Representative immunohistochemical stainings are shown in Fig. 1. The overexpression of Skp2 was undetectable in the normal epithelium (Fig. 1a). Skp2 staining was mainly detected in the cytoplasm and nuclei of squamous cell carcinoma cells (Fig. 1b, c). The nuclei of tumors were strongly stained. The overexpression of Skp2 was more frequent in OSCC (24 of 70, 34.3 %) than in the normal oral epithelium (0 of 10, 0 %; $p < 0.05$). It was also more frequently observed in cancers with higher grades according to the T classification (T1-2 vs T3-4; $p < 0.001$), N classification (N0 vs N1-2; $p < 0.05$), and pattern of invasion (grades 1-2 vs. grades 3-4; $p < 0.001$, Table 1). These results strongly suggested that

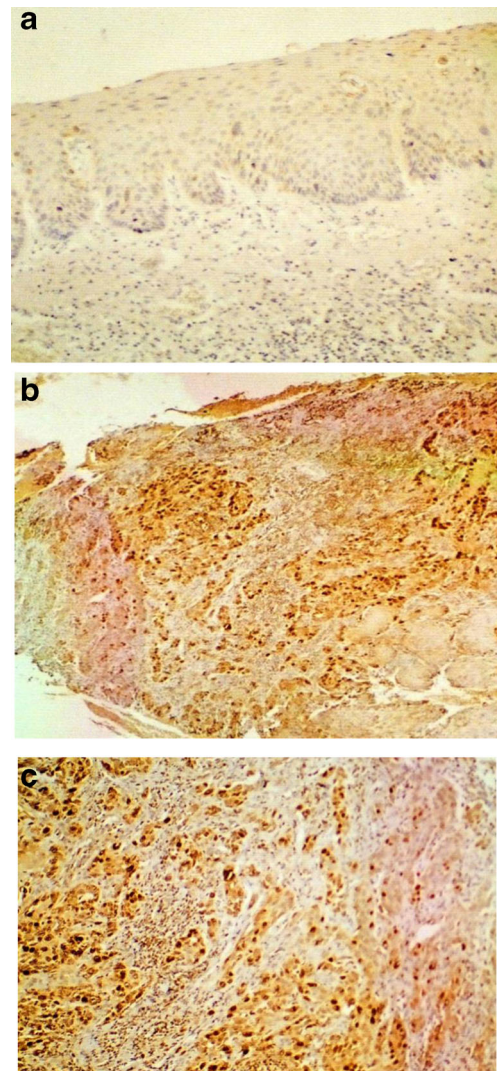


Fig. 1 Representative immunohistochemical staining for Skp2 in well-differentiated OSCC. **a** Immunohistochemical staining for Skp2 demonstrating the negative expression of Skp2 in the normal epithelium ($\times 40$). **b** Immunohistochemical staining for Skp2 demonstrating the strong cytoplasmic and nuclear expression of Skp2 ($\times 40$). **c** Immunohistochemical staining for Skp2 demonstrating the invasion front ($\times 100$)

the overexpression of Skp2 was a potent predictor of survival through progression and invasive potentials in OSCC patients.

Relationship between Skp2 Expression and the Survival Analysis

The 5-year overall survival rates of OSCC patients according to the expression of Skp2 were plotted (Fig. 2). The high-Skp2 expression group had a significantly poorer prognosis, at 30.1 %, than that of the low-expression group, at 63.0 % ($p < 0.05$). This result also strongly suggested that the overexpression of Skp2 was a potent predictor of survival, similar to the clinicopathological features described above.

Table 1 Relationships between the overexpression of Skp2 and clinicopathological features

Characteristics	Number of samples	Skp2 overexpression (-)	Skp2 overexpression (+)	<i>P</i> value
Normal epithelia	10	10	0	<i>P</i> < 0.05
SCCa	70	46	24	
Gender				0.689
Male	39	27	12	
Female	31	19	12	
Age				0.464
60	20	15	5	
>60	50	31	19	
T classification				<i>P</i> < 0.001
T1 + T2	46	36	10	
T3 + T4	24	10	14	
N classification				<i>P</i> < 0.05
N0	54	37	17	
N1 + N2	16	9	7	
Differentiation				0.695
well	60	38	22	
moderate/poor	10	8	2	
Pattern of Invasion				<i>P</i> < 0.001
Grades 1/2	43	34	9	
Grades 3/4	27	12	15	

Effects of Skp2 expression on migration and invasion potentials in HSC3 cells, an OSCC cell line

Cell migration and invasion are important and basic characteristics of tumor progression and metastasis. In order to determine the effects of Skp2 on the migration and invasion potentials of cells, we transfected HSC3 cells with Skp2 siRNA and performed wound healing and Matrigel invasion assays. Skp2 mRNA and protein levels were significantly lower in cells transfected with Skp2 siRNA than in non-transfected cells and cells transfected with scrambled siRNA (Fig. 3a, b). The healing rate was significantly lower, at 57.3 %, in cells in which the downregulation of Skp2 expression was induced than in controls 16 h after wounding (Fig. 3c). Concomitantly, the invasion index of HSC3 cells also significantly decreased from 94.1 % and 82.0 in cells

treated with vehicle alone and scrambled siRNA, respectively, to 39.6 % in those transfected with Skp2 siRNA (Fig. 3d). Therefore, the downregulation of Skp2 expression by siRNA markedly suppressed the mobility of HSC3 cells in vitro.

Effects of Decreasing Skp2 Expression on MMP-2 and MMP-9 Expression

Several MMPs have been reported to contribute to tumor growth by degrading the extracellular matrix, which promotes cell invasion, and by releasing growth factors to stimulate cell proliferation [31]. Among the MMP family, MMP-2 and MMP-9 have been shown to exhibit substrate specificity toward type IV collagen, which is the major component of the basement membrane [32]. In a previous study [33–35], an imbalance between MMP-2 and MMP-9 was shown to promote invasion and metastasis via Skp2 signaling pathways. Therefore, we examined the effects of Skp2 on the expression of MMP-2 and MMP-9 via Skp2 signaling pathways in the HSC3 cell line. The Skp2-targeted siRNA transfection of HSC3 cells led to reductions in the expression of MMP-2 and MMP-9 at the mRNA level (Fig. 4). Skp2-targeted siRNA transfection also decreased the expression of the transcription factor, Sp1. Moreover, the suppression of Skp2 reduced the enzyme activities of MMP-2 and MMP-9 (Fig. 5). These results suggested that the overexpression of Skp2 induced the upregulation of MMP-2 and MMP-9 via Sp1 and enhanced metastasis in OSCC cells.

Discussion

Several recent studies reported the clinicopathological and functional significance of Skp2 in various cancers. In non-small-cell lung cancer, increases in the expression of Skp2 correlated with lymph node metastasis, Stage II or a higher TNM classification, and poor or moderate differentiation [20]. In OSCC patients treated by UFT in combination with radiation therapy, a correlation was also reported between the expression of skp2 and tumor size, cervical lymph node metastasis, and patient outcomes [21]. The amplification and overexpression of Skp2 were previously shown to correlate with the tumor stage and lymph node metastasis in esophageal carcinoma [23]. In nasopharyngeal carcinoma, Skp2 was identified as a prognostic factor for a poor prognosis and maintained the cancer stem cell pool because it predicted high recurrence and migration risks at an early stage of the disease [24]. In the present study, we performed an immunohistochemical analysis in order to determine the relationships between the expression of Skp2 and clinicopathological features in OSCC patients. The results obtained revealed that the expression of skp2 correlated with the T classification, N classification, and particularly with the pattern of invasion, which

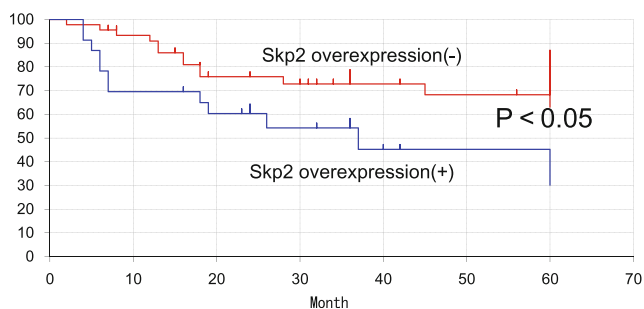


Fig. 2 Kaplan-Meier curves for a 5-year disease-specific survival analysis. The 5-year overall survival rates according to Skp2 expression in OSCC patients were plotted. The high-Skp2 expression group had a significantly poorer prognosis than that of the low-expression group ($p < 0.05$)

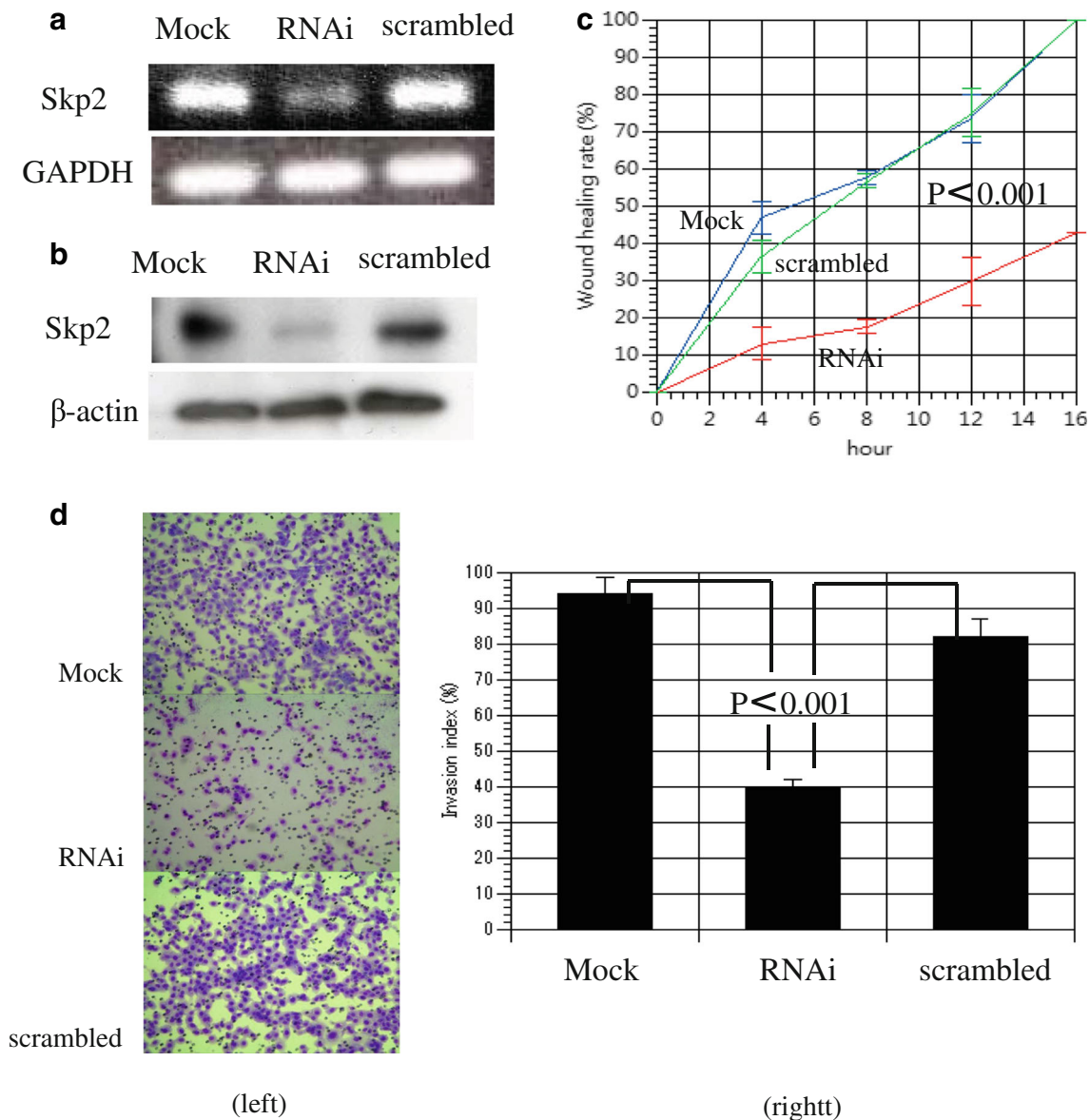


Fig. 3 RNAi of Skp2 in HSC3 cells. HSC3 cells were transfected with either scrambled or Skp2 siRNA. **a** After 72 h, isolated total RNA was analyzed by RT-PCR for Skp2 or GAPDH and **b** protein extracts were used in a western blotting analysis of Skp2 or β -actin. **c** The wound healing process was photographed 0, 4, 8, 12, and 16 h after wounding, and healing rates were determined as described in the Materials and

Methods section. The graph showed a significant decrease in the wound healing rate in HSC3 cells treated with Skp2 siRNA ($p < 0.001$). **(D)** The invasion of HSC3 cells (left) and percentage of invaded cells (right) were determined as described in the Materials and Methods section. The graph showed a significant decrease in the invasion index of HSC3 cells treated with Skp2 siRNA ($p < 0.001$)

was consistent with previous findings. Clinicopathologically, it has been suggested that the overexpression of Skp2 affects growth and invasion potentials in OSCC patients. The expression of Skp2 has also been associated with a poor prognosis in various cancers including gastric [19], head and neck [18, 21, 22, 24], and breast cancer [35] patients. The overall survival rate in this study also revealed that the Skp2 overexpression group had a poorer prognosis, at 30.1 %, than that of the low-expression group, at 63.0 %, for 5-year overall survival rates. Therefore, these results suggested that Skp2 expression levels were a useful prognostic factor in OSCC patients.

The function of Skp2 expression in cancers, including OSCC, currently remains unclear. Chan et al. demonstrated that the inactivation of Skp2 reduced cancer stem cell populations and their ability to form prostate spheres with genetic and pharmacological Skp2 reductions, suggesting that Skp2 is a previously uncharacterized key regulator for the maintenance of cancer stem cells [25]. In nasopharyngeal carcinoma, the knockdown of Skp2 partially reduced cell proliferation, promoted cellular senescence, and decreased the population of stem cell-like aldehyde dehydrogenase 1-positive cells as well as their self-renewal ability [24]. Furthermore, an inverse

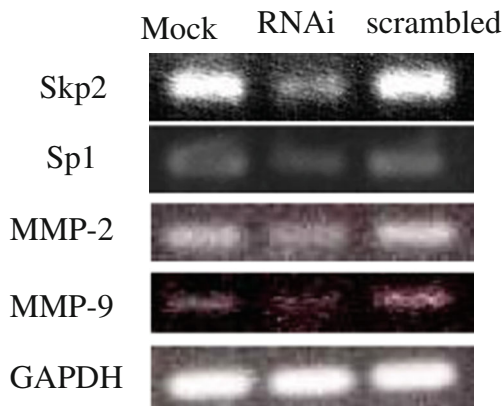


Fig. 4 Reduction in MMP-2 and MMP-9 by the suppression of Skp2 expression. HSC3 cells were transfected with either scrambled siRNA or Skp2 siRNA. After 72 h, proteins in whole cell lysates were analyzed by a RT-PCR analysis, which revealed that the expression of MMP-2, MMP-9, and Sp1 was decreased

correlation was reported between Skp2 and E-cadherin expression, which is a hallmark of EMT [36]. TGF- β 1 was also found to induce EMT partly through the upregulation of Skp2 [37]. EMT was previously found to play a role in the acquisition of resistance to paclitaxel in malignancies [38, 39]. In paclitaxel-resistant breast cancer cells, the acquisition of EMT has been associated with the expression of Skp2 [26]. The suppression of anoikis by the amplification and overexpression of Skp2 was previously reported to promote the metastasis of esophageal squamous cell carcinoma [23]. Since the prognosis of the high Skp2 expression group was poorer than that of the low expression group in the analysis of OSCC patients treated with UFT and radiation therapy, the functions of Skp2, including the positive regulation of cancer stem cell populations, suppression of anoikis, and acquisition of EMT through the expression of Skp2, may play crucial roles in OSCC patient survival.

The invasion and migration potentials of tumor cells are crucial in the process of metastasis. An *in vivo* assay in esophageal carcinoma cells revealed that the inhibition of Skp2 expression decreased tumor growth and lung metastasis [23]. In the invasion and migration assays conducted in the present study, the downregulation of Skp2 also significantly reduced invasion and migration potentials in HSC3 cells. The overexpression of Skp2 was previously reported to increase the expression of MMP-2 and MMP-9 via the transcriptional factor, Sp1 [33, 34]. An imbalance between MMP/tissue inhibitor of



Fig. 5 Downregulation of MMP-2 and MMP-9 enzyme activities by the suppression of Skp2 expression. Gelatin zymography revealed significant decreases in MMP-2 and MMP-9 enzyme activities in HSC3 cells treated with Skp2 siRNA

matrix metalloproteinase-1 (TIMP-1), including the upregulation of MMP-2 and MMP-9 and downregulation of TIMP-1, was identified as one of the mechanisms by which Skp2 promotes cell invasion. In the present study, Skp2-targeted siRNA transfection also decreased the expression of MMP-2, MMP-9, and the transcription factor, Sp1, in addition to decreasing MMP-2 and MMP-9 enzyme activities. These results also suggested that the overexpression of Skp2 induced the upregulation of MMP-2 and MMP-9 via Sp1 and enhanced the metastatic potential of OSCC cells. However, a co-treatment of MMP-2 and MMP-9 antibodies only reversed Skp2-enhanced invasion by 35–40 % [33, 34]. Therefore, Skp2 may promote cell invasion via other mechanisms [33, 34]. Further studies are needed to elucidate metastatic mechanisms through the expression of Skp2.

In conclusion, we herein demonstrated that Skp2 was associated with invasion and metastasis by upregulating migration and invasion potentials as well as the enzyme activities of MMP-2 and MMP-9 via Sp1, and may be a prognostic factor in OSCC patients. Further studies on the expression and function of Skp2 may offer additional indicators for the diagnosis and treatment of OSCC patients.

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Compliance with Ethical Standards

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Competing Interests None declared

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