

**Original**

**Quantification of (–)-epigallocatechin-3-gallate Inhibition of Migration and Invasion of Oral Squamous Cell Carcinoma Cell Lines Using Real-time Cell Analysis**

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**Abstract**: Catechins found in green tea, in particular (–)-epigallocatechin-3-gallate (EGCG), have antitumor activity. The primary antitumor actions of catechins are anti-oxidative, anti-angiogenic, and anti-metastatic effects. Cell migration and invasion contribute to the metastatic potential of tumors. Real-time cell analysis (RTCA) measures cell migration and invasion *in vitro*. In the present study, using RTCA, we investigated whether the cell migration and invasion of oral squamous cell carcinomas (OSCCs) of the tongue and floor of the mouth were inhibited by EGCG. Studies were performed using the human SCC-4 and SAS cell lines, which are poorly differentiated OSCCs of the tongue, and the HO-1-u-1 cell line, an OSCC of the floor of the mouth. SCC-4 cells exhibited high cell migration and invasion compared with the SAS and HO-1-u-1 cells. EGCG was most effective in inhibiting the migration and invasion of SCC-4 cells, and inhibited OSCC cell invasion more strongly than it inhibited cell migration. EGCG inhibited the expression of *matrix metalloproteinase (MMP)-2*, *MMP-9*, and *integrin  $\alpha 1$*  and  *$\beta 1$*  mRNA in the OSCC cell lines, particularly SCC-4 cells. The findings of the present study suggest that EGCG inhibits OSCC cell migration and invasion by inhibiting *MMP-2*, *MMP-9*, and *integrin  $\alpha 1$*  and  *$\beta 1$*  expression. Thus, EGCG may be a suitable agent or lead compound for the inhibition of OSCC metastasis.

**Key words**: oral squamous cell carcinoma, migration, invasion, (–)-epigallocatechin-3-gallate (EGCG), real-time cell analyzer

**Introduction**

Green tea is a popular beverage in Japan. Because tea contains catechins and polyphenols, it may have potential in preventing cancer<sup>1)</sup>. A recent study reported a tendency for a reduced risk of oral cancer in women who consumed green tea<sup>2)</sup>. Catechins are an important constituent of green tea and include (–)-epigallocatechin-3-gallate (EGCG), (–)-epigallocatechin, (–)-epicatechin-3-gallate, and (–)-epicatechin<sup>3)</sup>. Catechins, especially EGCG, exhibit notable bioactivity in

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green tea extracts, including antitumor activity and anti-oxidative and anti-angiogenic effects<sup>4, 5</sup>). Moreover, EGCG acts at numerous points to regulate metastasis, including effects at the DNA, RNA, and protein levels<sup>6</sup>).

Oral squamous cell carcinoma (OSCC) is the most common malignancy of the oral cavity and has a high incidence of cervical micrometastases<sup>7</sup>). Distant metastases reduce patient quality of life and affect the clinical outcome<sup>7, 8</sup>). Increased tumor size and microvascular invasion are the most significant independent prognostic factors in predicting the survival of patients with OSCC<sup>9</sup>). Therefore, the presence of metastasis and invasion are the main prognostic factors of patients with OSCCs.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent proteinases that are strongly related to cancer metastasis because of their association with extracellular matrix (ECM) degradation and cellular migration<sup>10</sup>). In particular, MMP-2 and MMP-9 are highly expressed in OSCC compared with normal oral mucosal tissues, and their mRNA and protein levels are further increased upon tumor progression<sup>11, 12</sup>). In addition, it is well established that MMP-2 and MMP-9 are closely associated with tumor invasion and metastasis in a variety of human tumors<sup>13, 14</sup>). In a previous study, we reported that EGCG inhibited the mRNA and protein expression and activity of MMPs in human umbilical vein endothelial cells co-cultured with fibroblasts or osteoclasts<sup>15, 16</sup>). Moreover, EGCG inhibits carcinoma cell migration and invasion via MMP-2 and MMP-9<sup>17</sup>). Integrins are transmembrane heterodimeric receptors that interact with the ECM. Expression of integrins and proteases of the MMP and plasminogen activator families is altered in both healing and malignant epithelium, and is thus thought to be key in the process of re-epithelialization, cell migration, and tumor invasion<sup>18, 19</sup>). EGCG also inhibits the activation and expression of integrins<sup>17</sup>). It is therefore thought that EGCG plays an important role in inhibition of cancer metastasis by inhibiting MMPs and integrins.

Previously, we reported on determination of the migration and invasion of OSCCs using the xCELLigence real-time cell analysis (RTCA) system (Roche Applied Science, Mannheim, Germany)<sup>14</sup>). This system makes use of impedance detection for continuous monitoring of cell viability, migration, and invasion<sup>20</sup>). In the present study, we used RTCA to investigate EGCG inhibition of the migration and invasion of OSCCs of the tongue and floor of the mouth, as well as the expression of associated *MMP* and *integrin* mRNA.

## Materials and methods

### *Cell lines and reagents*

The SCC-4 (JCRB9118)<sup>21</sup>) and SAS (JCRB0260)<sup>22</sup>) cell lines were both established from poorly differentiated OSCC of the tongue. The HO-1-u-1 cell line (JCRB0828) was established from a poorly differentiated OSCC in the floor of the mouth<sup>23</sup>). All three cell lines were provided by the Health Science Research Resources Bank (Osaka, Japan). EGCG was purchased from Wako Pure Chemical Industries (Osaka, Japan).

### *Cell culture*

SAS and HO-1-u-1 cells were cultured at 37°C under a humidified 5% CO<sub>2</sub> and 95% air atmosphere in 45% Dulbecco's modified Eagle's medium with 45% Ham's F12 medium (DMEM/F12), 10% fetal calf serum (FCS), 100 U/ml penicillin, and 100 µg/ml streptomycin. SCC-4 cells, which are undifferentiated cancer cells, were cultured under the same conditions in DMEM/F12 with 0.4 µg/ml hydrocortisone, 10% FCS, 100 U/ml penicillin, and 100 µg/ml streptomycin. The culture medium for all cells was changed every 3 days.

### *Real-time cell analysis*

The migration and invasion of OSCC cells were analyzed by RTCA, as described previously<sup>14, 24</sup>. Migration was assessed on an RTCA system CIM-Plate 16 coated with fibronectin (20 mg/ml). SCC-4 cells ( $1.6 \times 10^5$ ) or  $3.2 \times 10^5$  SAS or HO-1-u-1 cells were seeded into the upper chamber of individual CIM-Plate 16s in 100 µl medium specific for each cell line without FCS, and with or without EGCG (10 or 100 µM). The upper chamber was then placed on the lower chamber of the CIM-Plate 16, which contained growth medium supplemented with 10% FCS as an attractant. Changes in impedance resulting from cells that had migrated to the bottom side of the membranes were recorded every 15 min and were monitored for a total of 48 hr.

Invasion was assessed using a CIM-plate 16 coated with Matrigel™ (1:80; BD Biosciences, Erembodegem, Belgium) and fibronectin (20 mg/ml). SCC-4 cells ( $1.6 \times 10^5$ ) or  $3.2 \times 10^5$  SAS or HO-1-u-1 cells were seeded in the upper chamber of individual CIM-Plate 16s in 100 µl medium specific for each cell line without FCS, and with or without EGCG (10, 20, or 100 µM). The upper chamber was then placed on the lower chamber of the CIM-Plate 16 containing growth medium supplemented with 10% FCS as an attractant. Changes in impedance resulting from cells that had migrated to the bottom side of the membranes were recorded every 15 min and were monitored for a total of 48 hr.

A unitless parameter termed cell index was used to measure the relative change in electrical impedance as a marker of cell status. The cell index is a relative and dimensionless value because it represents the impedance change divided by a background value.

### *RNA isolation and quantitative real-time reverse transcription-polymerase chain reaction*

OSCC cells were seeded in six-well plates, grown for 1 week at 37°C, and then treated with EGCG (0, 10 and 100 µM) for 5 hr. Quantitative polymerase chain reaction (qPCR) was used to quantify mRNA levels of *MMP-2*, *MMP-9*, and *integrin α1* and *β1* in OSCC cells. Total RNA was extracted using NucleoSpin RNA II (MACHEREY-NAGEL, Duren, Germany) and reverse transcribed using the PrimeScript RT Master Mix (TaKaRa Bio, Shiga, Japan). The resulting cDNAs were amplified using primers designed using ProbeFiber software (Roche). Table 1 lists the primers used, the Roche Universal ProbeLibrary probe numbers, and the gene accession numbers. Amplification was performed with a LightCycler (Roche) using the LightCycler TaqMan Master mix (Roche). The PCR reaction parameters were as follows: 95°C for

Table 1. Nucleotide sequences of primers used for polymerase chain reaction

Gene	Sequence	Accession no.	Probe no.
<i>MMP-2</i>	Sense : 5'-gagggggcaggtag-3'	AY738117.1	#58
	Antisense : 5'-ggctggatgagatcttgctg-3'	AY738117.1	
<i>MMP-9</i>	Sense : 5'-gaaccaatctcaccgacagg-3'	NM_004994.2	#6
	Antisense : 5'-gccaccgagtgtaaccata-3'	NM_004994.2	
<i>Integrin <math>\alpha 1</math></i>	Sense : 5'-aattggctctagtcaccattgtt-3'	NM_181501.1	#14
	Antisense : 5'-caaatgaagctgctgactggt-3'	NM_181501.1	
<i>Integrin <math>\beta 1</math></i>	Sense : 5'-cgatgccatcatgcaagt-3'	NM_002211.3	#65
	Antisense : 5'-acaccagcagccgtgaac-3'	NM_002211.3	
<i>18s</i>	Sense : 5'-gcaattattcccatgaacg-3'	X03205.1	#48
	Antisense : 5'-gggactaatcaacgaagc-3'	X03205.1	

10 min, followed by 45 cycles (except for *18s* rRNA amplification = 25 cycles) of 10 sec at 95°C, 30 sec at 60°C, and 1 sec at 72°C. Fluorescence data were analyzed using LightCycler software (Roche). mRNA levels of the genes of interest were normalized against those of *18s* rRNA used as an internal standard to yield relative expression ratios<sup>14)</sup>.

#### Statistical analysis

The normal distribution of baseline variables for all RTCA data was evaluated using the Bonferroni test following one-way analysis of variance (ANOVA). PCR data were analyzed using the two-sided Mann-Whitney *U*-test. All data are expressed as the mean  $\pm$  SEM.  $P < 0.05$  was considered significant.

#### Results

Fig. 1 shows the results of RTCA of the effect of EGCG on cell invasion through Matrigel<sup>TM</sup>- and fibronectin-coated invasion chambers. EGCG was added to the upper chamber of the invasion apparatus at a concentration of 10 or 100  $\mu$ m and inhibited the invasion of OSCCs in a concentration-dependent manner. After 18 hr incubation, the cell index for control, 10, and 100  $\mu$ m EGCG-treated SCC-4 cells was  $3.66 \pm 0.10$  ( $n = 10$ ),  $0.17 \pm 0.12$  ( $n = 7$ ), and  $0.02 \pm 0.02$  ( $n = 7$ ), respectively. EGCG treatment resulted in an approximate 100% inhibition of cell invasion for up to 18 hr after EGCG addition (Fig. 1A). At 48 hr after EGCG addition, the invasion of SCC-4 cells was inhibited by 98.7% in the presence of 100  $\mu$ m EGCG ( $P < 0.01$  vs control and 10  $\mu$ m EGCG) and by 76.3% in the presence of 10  $\mu$ m EGCG ( $P < 0.01$  vs control; Fig. 1A). The invasion of SAS cells was decreased by 93.2% after 24 hr exposure to 100  $\mu$ m EGCG ( $P < 0.01$  vs control and 10  $\mu$ m EGCG) and by 63.4% after 48 hr exposure to 10  $\mu$ m EGCG ( $P < 0.01$  vs control;  $n = 10$  wells [control] and 6 wells [10 and 100  $\mu$ m EGCG treatment]; Fig. 1B). The invasion of HO-1-u-1 cells was decreased by 92.7% after 24 hr

exposure to 100  $\mu\text{m}$  EGCG ( $P < 0.05$  vs control and 10  $\mu\text{m}$  EGCG) and by 57.4% after 48 hr exposure to 10  $\mu\text{m}$  EGCG ( $P < 0.01$  vs control;  $n = 10$  wells [control] and 5 wells [10 and 100  $\mu\text{m}$  EGCG]; Fig. 1C).

Fig. 2 shows the effect of EGCG on cell migratory responses on a fibronectin substrate as determined using RTCA. EGCG was added to the upper chamber of the migration apparatus at a concentration of 10, 20, or 100  $\mu\text{m}$ . The migration of SCC-4 cells was decreased by 99.9% following 48 hr treatment with 100  $\mu\text{m}$  EGCG ( $P < 0.01$  vs control and 10 and 20  $\mu\text{m}$  EGCG) and by 55.1% after 48 hr exposure to 10  $\mu\text{m}$  EGCG ( $P < 0.01$  vs control;  $n = 10$  wells [control] and 7 wells [10, 20, and 100  $\mu\text{m}$  EGCG]; Fig. 2A). The migration of SAS cells decreased by 95.7% following 48 hr treatment with 100  $\mu\text{m}$  EGCG ( $P < 0.01$  vs control and 10 and 20  $\mu\text{m}$  EGCG) and by 16.6% after 48 hr exposure to 10  $\mu\text{m}$  EGCG ( $P < 0.01$  vs control and 20 and 100  $\mu\text{m}$  EGCG;  $n = 10$  wells [control], 6 wells [10 and 100  $\mu\text{m}$  EGCG], and 7 wells [20  $\mu\text{m}$  EGCG]; Fig. 1B). The migration of HO-1-u-1 cells was decreased by 93.9% following 48 hr treatment with 100  $\mu\text{m}$  EGCG ( $P < 0.01$  vs control and 10 and 20  $\mu\text{m}$  EGCG), but there was no significant difference in migration between the control and 10  $\mu\text{m}$  EGCG-treated groups after 48 hr ( $n = 10$  wells [control], 5 wells [10 and 100  $\mu\text{m}$  EGCG], and 7 wells [20  $\mu\text{m}$  EGCG]; Fig. 2C). In the case of SCC-4 cells, the effects of EGCG on the invasion response were similar to its effects on the migratory response. However, in the case of SAS and HO-1-u-1 cells, EGCG induced a greater decrease in cell invasion than cell migratory responses, particularly at a concentration of 10  $\mu\text{m}$ .

Fig. 3 and 4 show *MMP-2* and *MMP-9* mRNA expression in OSCCs after 5 hr exposure to EGCG. In these experiments, control expression was set at 100%. Treatment of SCC-4 cells with 100  $\mu\text{m}$  EGCG induced a 52.9% decrease in *MMP-2* mRNA expression compared with control ( $P < 0.05$ ;  $n = 6$  for all; Fig. 3A). However, EGCG had no significant effect on *MMP-2* mRNA levels in SAS and HO-1-u-1 cells ( $n = 4$  for all; Fig. 3B, C). Treatment of SCC-4 cells with 10 and 100  $\mu\text{m}$  EGCG resulted in a 60.7% and 67.0% decrease in *MMP-9* mRNA levels, respectively, compared with control ( $P < 0.05$  for both;  $n = 6$  for all; Fig. 4A). Similarly, EGCG (10 and 100  $\mu\text{m}$ ) treatment of HO-1-u-1 cells decreased *MMP-9* mRNA expression by 43.8% and 55.0%, respectively, compared with control ( $P < 0.05$  for both;  $n = 4$  for all; Fig. 4C). There was a tendency for *MMP-9* mRNA levels to decrease in SAS cells following EGCG treatment compared with control ( $n = 4$  for all; Fig. 4B).

Fig. 5 and 6 show *integrin  $\alpha 1$*  and  *$\beta 1$*  mRNA expression in OSCC cells after 5 hr treatment with EGCG. EGCG treatment (100  $\mu\text{m}$ ) of SCC-4 (Fig. 5A;  $n = 4$ ) and HO-1-u-1 (Fig. 5C;  $n = 6$ ) cells decreased *integrin  $\alpha 1$*  mRNA levels to 32.6% and 45.5% of control, respectively ( $P < 0.05$  for both). There was a tendency for decreased *integrin  $\alpha 1$*  mRNA expression in 100  $\mu\text{m}$  EGCG-treated SAS cells compared with control ( $n = 4$  for all; Fig. 5B). *Integrin  $\beta 1$*  mRNA levels in SCC-4 cells were decreased by 58.5% and 60.4% compared with control following treatment with 10 and 100  $\mu\text{m}$  EGCG, respectively ( $P < 0.01$  for both;  $n = 6$ ; Fig. 6A). Treatment of SAS and HO-1-u-1 cells with 100  $\mu\text{m}$  EGCG decreased *integrin  $\beta 1$*  mRNA levels by 64.7% and 69.8%, respectively, compared with control ( $P < 0.05$  for both;  $n = 4$  for all; Fig. 6B, C).

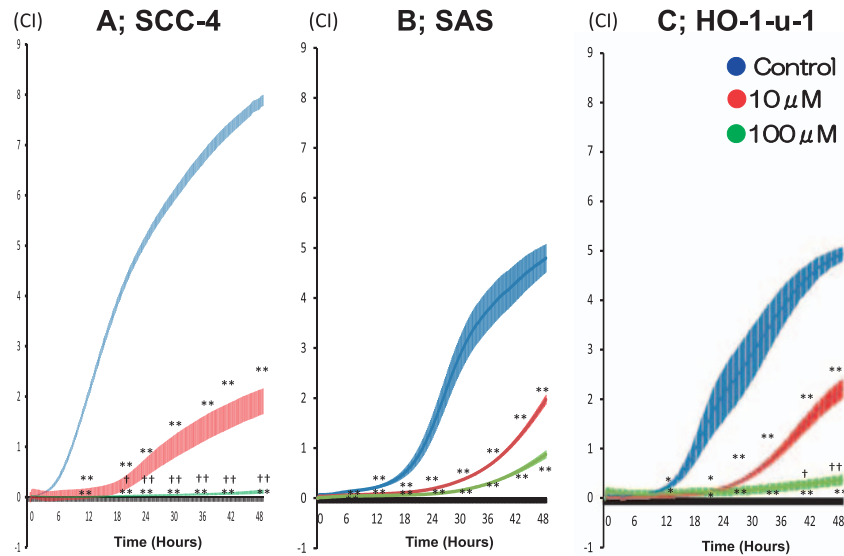


Fig. 1. EGCG inhibition of invasion

Invasion of (A) SCC-4, (B) SAS, and (C) HO-1-u-1 cell lines through Matrigel™ and fibronectin was analyzed using real-time cell analysis. Cells were seeded into wells of a CIM-Plate 16 ( $1.6 \times 10^5$  SCC-4 cells/well;  $3.2 \times 10^5$  SAS and HO-1-u-1 cells/well) and were allowed to migrate in the absence (Control) or presence of 10 or 100  $\mu\text{M}$  (-)-epigallocatechin-3-gallate (EGCG) added to the upper compartment of each well. Data are the mean  $\pm$  SEM cell index (CI) of  $n$  wells ( $n=10$  [control] and 7 [10 and 100  $\mu\text{M}$  EGCG] in A;  $n=10$  [control] and 6 [10 and 100  $\mu\text{M}$  EGCG] in B; and  $n=10$  [control] and 5 [10 and 100  $\mu\text{M}$  EGCG] in C). \* $P < 0.05$ , \*\* $P < 0.01$  compared with control; † $P < 0.05$ , †† $P < 0.01$  compared with 10  $\mu\text{M}$  EGCG.

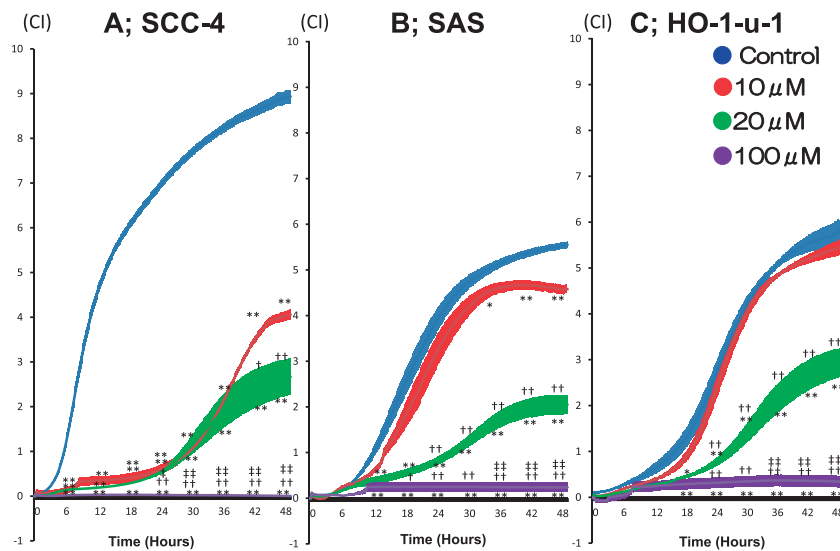


Fig. 2. EGCG inhibition of migration

Migration of (A) SCC-4, (B) SAS, and (C) HO-1-u-1 cell lines over fibronectin was analyzed using real-time cell analysis. Cells were seeded into wells of a CIM-Plate 16 ( $1.6 \times 10^5$  SCC-4 cells/well;  $3.2 \times 10^5$  SAS and HO-1-u-1 cells/well) and were allowed to migrate in the absence (Control; blue) or presence of 10 (red), 20 (green), or 100  $\mu\text{M}$  (purple) (-)-epigallocatechin-3-gallate (EGCG) added to the upper compartment of each well. Data are the mean  $\pm$  SEM cell index (CI) of  $n$  wells ( $n=10$  [control] and 7 [10, 20, and 100  $\mu\text{M}$  EGCG] in A;  $n=10$  [control], 6 [10 and 100  $\mu\text{M}$  EGCG], and 7 [20  $\mu\text{M}$  EGCG] in B; and  $n=10$  [control], 5 [10 and 100  $\mu\text{M}$  EGCG], and 7 [20  $\mu\text{M}$  EGCG] in C). \* $P < 0.05$ , \*\* $P < 0.01$  compared with control; † $P < 0.05$ , †† $P < 0.01$  compared with 10  $\mu\text{M}$  EGCG; ‡ $P < 0.05$ , ‡‡ $P < 0.01$  compared with 20  $\mu\text{M}$  EGCG.

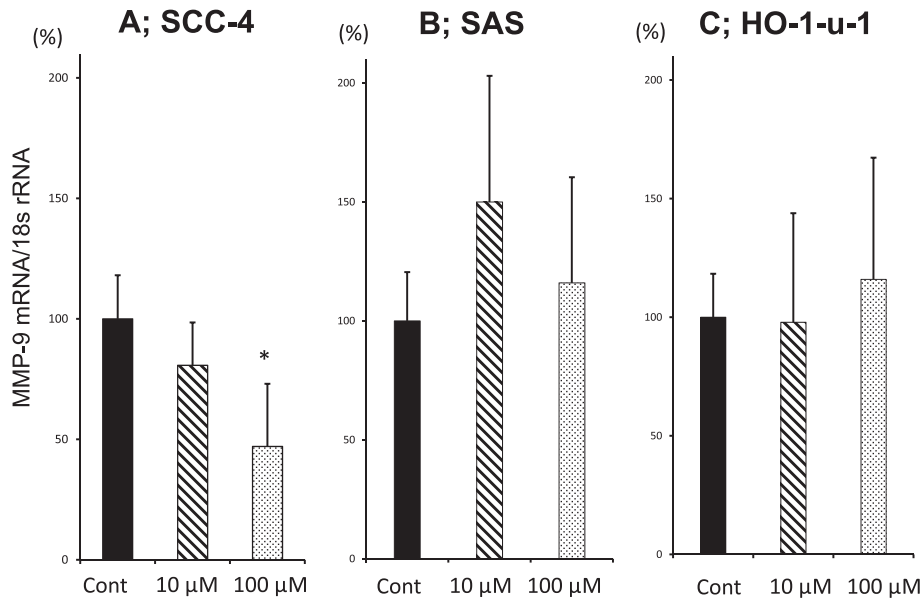


Fig. 3. Effect of EGCG on *MMP-2* mRNA expression of oral squamous cell carcinoma cells *Matrix metalloproteinase (MMP)-2* mRNA expression in (A) SCC-4, (B) SAS, and (C) HO-1-u-1 cell lines, as determined by quantitative polymerase chain reaction. Expression of *MMP-2* mRNA was normalized against that of *18s* rRNA, used as an internal control. Expression in the control group was set at 100% and expression in the remaining groups is shown relative to the control group. Data are the mean  $\pm$  SEM ( $n=6$  for all groups in A;  $n=4$  for all groups in B and C). \* $P < 0.05$  compared with control.

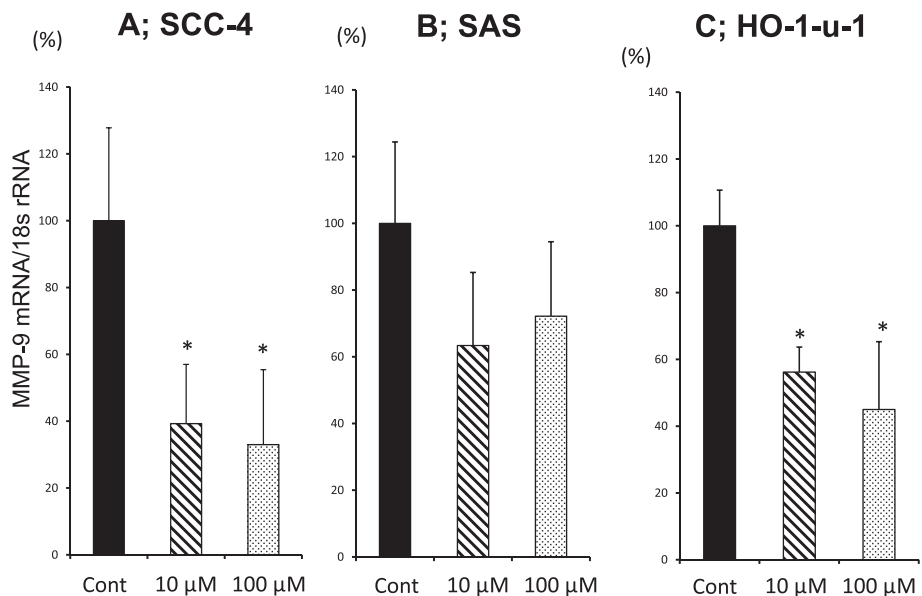


Fig. 4. Effect of EGCG on *MMP-9* mRNA expression of oral squamous cell carcinoma cells *Matrix metalloproteinase (MMP)-9* mRNA expression in (A) SCC-4, (B) SAS, and (C) HO-1-u-1 cell lines, as determined by quantitative polymerase chain reaction. Expression of *MMP-9* mRNA was normalized against that of *18s* rRNA, used as an internal control. Expression in the control group was set at 100% and expression in the remaining groups is shown relative to the control group. Data are the mean  $\pm$  SEM ( $n=6$  for all groups in A;  $n=4$  for all groups in B and C). \* $P < 0.05$  compared with control.

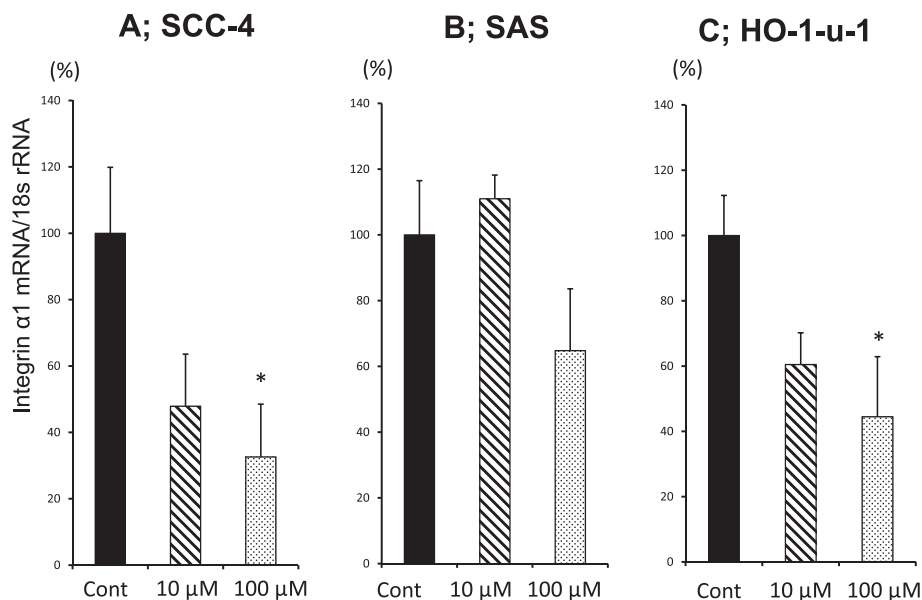


Fig. 5. Effect of EGCG on *integrin  $\alpha 1$*  mRNA expression of oral squamous cell carcinoma cells. Integrin  $\alpha 1$  mRNA expression in (A) SCC-4, (B) SAS, and (C) HO-1-u-1 cell lines, as determined by quantitative polymerase chain reaction. Expression of *integrin  $\alpha 1$*  mRNA was normalized against that of *18s* rRNA, used as an internal control. Expression in the control group was set at 100% and expression in the remaining groups is shown relative to the control group. Data are the mean  $\pm$  SEM (n=6 for all groups in A ; n=4 for all groups in B and C). \* $P < 0.05$  compared with control.

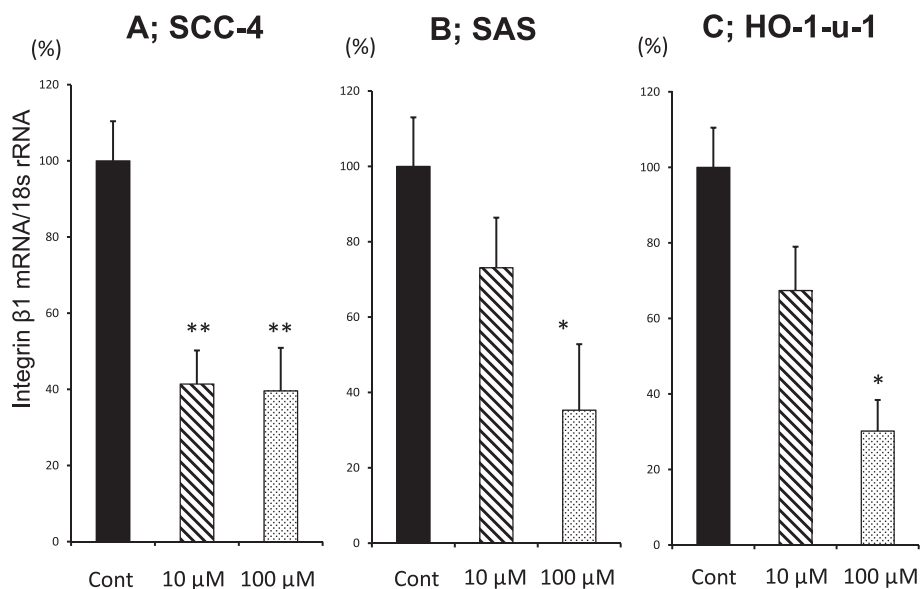


Fig. 6. Effect of EGCG on *integrin  $\beta 1$*  mRNA expression of oral squamous cell carcinoma cells. Integrin  $\beta 1$  mRNA expression in (A) SCC-4, (B) SAS, and (C) HO-1-u-1 cell lines, as determined by quantitative polymerase chain reaction. Expression of *integrin  $\beta 1$*  mRNA was normalized against that of *18s* rRNA, used as an internal control. Expression in the control group was set at 100% and expression in the remaining groups is shown relative to the control group. Data are the mean  $\pm$  SEM (n=6 for all groups in A ; n=4 for all groups in B and C). \* $P < 0.05$ , \*\* $P < 0.01$  compared with control.



## Discussion

In the present study, using RTCA, we demonstrated that EGCG inhibited the migratory and invasion responses of poorly differentiated OSCC cell lines and that these responses were associated with changes in *MMP-9* and *integrin  $\alpha 1$*  in real time. The use of RTCA facilitated the capture of the effects of EGCG on each of the three types of OSCC cells.

A frequent problem associated with the use of EGCG as an anticancer agent is its low blood concentrations. Lee *et al.* reported that locally high concentrations of tea polyphenols ( $C_{\max} = 131.0\text{--}2.2 \mu\text{m}$ ) could be achieved by holding green tea leaves in the oral cavity<sup>25</sup>). In the present study, using RTCA, we demonstrated that  $100 \mu\text{m}$  EGCG clearly inhibits cell migratory and invasion responses; these findings suggest that EGCG has the potential to be effective against cancer cells. However, it will be difficult to immediately use EGCG as a clinical treatment because the route of administration and dosage need to be established.

MMP-2 and MMP-9 are important targets for the prevention of cancer migration and invasion<sup>26</sup>). This is because the cancer cell-matrix interaction is a critical step in the promotion of cell migration<sup>27</sup>), and proteolytic degradation of the ECM is a crucial event during tumor invasion and metastasis. This role of MMPs explains why the invasion and migration of OSCC cell lines were inhibited by EGCG-induced downregulation of MMP-2 and MMP-9 production in the present study. In a previous study, using the broad-spectrum MMP inhibitor marimastat, we demonstrated that MMPs are associated with the invasion and metastasis of OSCCs<sup>14</sup>). Moreover, it is known that EGCG inhibits the mRNA expression and activity of MMP-2 and MMP-9<sup>15, 16</sup>). SCC-4 cells expressed much higher levels of MMP than SAS and HO-1-u-1 cells. It is therefore interesting that MMP-2 and MMP-9 mRNA levels and the tumor invasion and migration of SCC-4 cells were more strongly inhibited by EGCG than those of SAS and HO-1-u-1 cells. The differences in the inhibitory effects of EGCG on the different cell lines were particularly strong for the effects of  $10 \mu\text{m}$  EGCG on tumor invasion and migration. Moreover, in the present study EGCG inhibited the invasion of the OSCC cell lines more effectively than it inhibited their migration. The greater effect of EGCG on cell invasion, as determined using the RTCA, may be due to the fact that MMP-2 and MMP-9 appear to be necessary for degradation of the Matrigel<sup>TM</sup> during tumor invasion. No effect of EGCG on *MMP-2* mRNA expression in SAS or HO-1-u-1 cells was observed. However, EGCG has been shown previously to affect MMP activity, in particular MMP-2 activity, both directly and indirectly at relatively low doses ( $10\text{--}20 \mu\text{m}$ )<sup>16</sup>).

The  $\alpha$ - and  $\beta$ -subunits of integrins contribute to the formation of the ligand-binding pocket. However, the  $\alpha$ -subunit is likely to play an important role in determining binding selectivity because integrin heterodimers often share a common  $\beta$ -subunit but have a distinct  $\alpha$  chain and bind different ligands<sup>28</sup>). Coordinated cellular responses to ECM attachment that are mediated by integrins have been shown to induce tumor cell metastasis<sup>29</sup>). Sen and Chatterjee reported that integrin-mediated signaling is necessary for epidermal growth factor (EGF)-mediated responses and that integrins collaborate with EGF in the phosphorylation and enhancement

of activation of tyrosine kinases, such as focal adhesion kinase (FAK) and extracellular signal-regulated kinase (ERK)<sup>30</sup>. EGCG inhibits the cooperative responses of integrin and growth factor receptors<sup>30</sup>. In the present study, we showed that EGCG reduced *integrin  $\alpha 1$*  and  *$\beta 1$*  mRNA expression in OSCC cell lines.

In conclusion, using RTCA technology, we clearly showed EGCG-induced inhibition of the migratory and invasion responses of poorly differentiated OSCC cell lines in real time. The results suggest that EGCG may be a suitable agent or lead compound for the inhibition of metastases of OSCCs by inhibiting MMPs and integrins.

#### Conflict of interest disclosure

The authors have declared no conflict of interest.

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[Received January 14, 2015 : Accepted January 26, 2015]