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Original

Quantification of Cell Migration and Invasion, and Their Association with Periostin in Anaplastic Thyroid Cancer, Using a Real-time Cell Analyzer

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Abstract: Anaplastic thyroid cancer (ATC) is known to be a highly malignant cancer of the thyroid with a high mortality rate. In a previous study, we used real-time cell analysis (RTCA) to analyze cell migration and invasion of oral squamous cell carcinomas (OSCCs) of the tongue and floor of the mouth. In the present study, we investigated cell migration and invasion of ATC using RTCA, as well as their association with periostin, matrix metalloproteinases (MMPs), and integrins. Experiments were performed on TCO-1 and HTC/C3 cells, which are human ATC cell lines. OSCC cell lines were used for comparison. Using the cell analysis system, cell migration was assessed on fibronectin-coated CIM-Plates, whereas invasion was assessed on fibronectin- and matrigel-coated CIM-Plates. SCC-4 cells exhibited high cell migration and invasion activity compared with other OSCC cell lines. TCO-1 cells exhibited equivalent cell invasion but stronger migration than SCC-4 cells. Although TCO-1 cells had strong invasive activity, they did not express MMP-9, unlike SCC-4 cells. Conversely, periostin expression was high in TCO-1 cells. Therefore, periostin expression appears to be associated with the cell migration and invasion activity of ATC. The RTCA system will be useful for the analysis of the metastatic characteristics of ATC in head and neck cancer.

Key words: anaplastic thyroid cancer, migration, invasion, periostin, real-time cell analyzer

Introduction

Anaplastic thyroid cancer (ATC) is a rare and highly malignant cancer of the thyroid with a high mortality rate[1)]. Only a very small percentage of patients undergo surgical intervention for ATC[2)]. Treatment of ATC is very difficult and the survival rate is the lowest for those with head and neck cancer[1, 3)]. Epithelial–mesenchymal transition is typical of dedifferentiated cancers such as ATC, and is associated with high invasion and dissemination potential[4)].

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The xCELLigence real-time cell analysis (RTCA) system (SCRUM Inc., Tokyo, Japan) was developed for evaluation of cell migration and invasion *in vitro*[5]. This system uses impedance detection to produce a cell index for the continuous monitoring of cell viability, migration, and invasion[6]. Previously, we reported on the migration and invasion of oral squamous cell carcinomas (OSCCs) using the RTCA system[5]. That study indicated that SCC-4 cells (tongue cell line) in particular exhibit strong cell migration and invasion in association with the expression of matrix metalloproteinase (MMP)-9[5]. MMPs are a family of zinc-dependent proteinases, and activated MMPs degrade many extracellular matrix (ECM) and basement membrane components[7, 8]. MMPs play a role in cancer proliferation and metastasis because of their role in ECM degradation, cellular migration, tissue remodeling, and angiogenesis[9]. In particular, MMP-2 and MMP-9 are closely associated with tumor invasion and metastasis in a variety of human tumors[10–12]. MMP-7 also has an important role in the metastasis of ATC[13]. Tissue-specific inhibitors of metalloproteinases (TIMPs) are tissue-specific endogenous inhibitors of metalloproteinases, including the MMPs. There are four homologous members of the TIMP family: TIMP-1, TIMP-2, TIMP-3, and TIMP-4[14]. TIMPs tightly control MMP activity in a 1 : 1 ratio[15]. TIMP-1, TIMP-2, and TIMP-4 are soluble, whereas TIMP-3 is closely associated with the ECM. Generally, all TIMPs are capable of inhibiting all known MMPs; however, TIMP-2, TIMP-3 and TIMP-4 are capable of interaction with pro-MMP-2[14, 15].

Humans integrins have 18α- and eight β-subunits that combine to give 24 different $\alpha\beta$ heterodimers[16]. $\alpha\beta$ Integrin heterodimers are transmembrane heterodimer receptors that interact with the ECM. Integrins coordinate the interaction of a cell with the ECM by associating on the cell membrane with other proteins and by signaling to modify the expression of molecules such as matrix-modifying proteolytic enzymes[17]. Thus, integrins permit dynamic bidirectional transmembrane signaling that is essential in cell adhesion, migration, differentiation, and survival.

Periostin was originally identified as an 811-amino acid protein secreted by osteoblasts. It shares structural homology with insect fasciclin I and can bind heparin and support the adhesion of osteoblasts[18]. Periostin is a modular glycoprotein frequently observed to be a major constituent of the extracellular milieu of desmoplastic malignant tumors[19]. Gillan *et al* discussed that periostin functions as a ligand for $\alpha_V\beta_3$ and $\alpha_V\beta_5$ integrins to support the adhesion and migration of ovarian epithelial cells[18].

In the present study we investigated cell migration and invasion using the RTCA system and their association with MMPs, TIMPs, integrins, and periostin in ATC compared with OSCCs of the tongue and floor of the mouth.

Materials and methods

Cell lines and reagents

All cell lines used in the present study were provided by the Health Science Research Resources Bank (Osaka, Japan). TCO-1 (JCRB0239) cells were established from a human non-differentiated thyroid cancer[20], whereas HTC / C3 (JCRB0164) cells are a human thyroid

carcinoma cell line[21]. T.T (JCRB0262) cells are a human squamous cell carcinoma (SCC) cell line derived from esophageal cancer, KD (JCRB9103) cells are human fibroblasts of the lip[22], and SCC-4 (JCRB9118) and SAS (JCRB0260) cells were established from a poorly differentiated OSCC in the tongue. HO-1-u-1 (JCRB0828) cells were established from a poorly differentiated OSCC in the floor of the mouth[5].

Cell culture

TCO-1, HTC / C3, and KD cells were cultured in Dulbecco's modified Eagle's medium (DMEM), whereas SAS, HO-1-u-1, and T.T cells were cultured in 45% DMEM + 45% Ham's F12 medium (DMEM / F12). SCC-4 cells are considered undifferentiated cancer cells and were cultured under the same conditions in DMEM / F12 with 0.4 μg / ml hydrocortisone. All cell lines were cultured at 37°C in humidified 5% CO_2 and 95% air, and each medium was supplemented with 10% fetal bovine serum (FBS), 100 U / ml penicillin, and 100 μg / ml streptomycin. The medium was changed every 3 days.

RTCA

RTCA was used in the present study to evaluate the migration and invasion of head and neck cancer cell lines[5, 23]. Migration was analyzed on a CIM-Plate 16 that was coated with fibronectin (20 mg / ml), whereas invasion was assessed using a CIM-Plate 16 coated with matrigel (1 : 80 ; BD Biosciences, Erembodegem, Belgium) and fibronectin (20 mg / ml). In both cases, cells from each cell line (TCO-1 and SCC-4 cells, 1.6×10^5 cells / well ; HTC / C3, SAS, HO-1-u-1, T.T and KD cells, 3.2×10^5 cells / well) were seeded in the upper chamber of a CIM-Plate 16 in 100 μl medium without FBS. The upper chamber was then placed on the lower chamber of the CIM-Plate 16, which contained growth medium supplemented with 10% FBS as an attractant.

RNA isolation and quantitative real-time RT-PCR (qPCR)

In the present study, qPCR was used to quantify mRNA levels of *MMP-2*, *MMP-7*, *MMP-9*, *MMP-14*, *TIMP-1*, *TIMP-2*, *TIMP-3*, *TIMP-4*, α_1 integrin (*ITGA1*), *ITGAV*, *ITGB5*, and periostin in OSCCs. Total RNA was extracted using NucleoSpin RNA II (MACHEREY-NAGEL, Duren, Germany), and reverse transcription of total RNA was performed using PrimeScript RT Master Mix from TaKaRa Bio (Shiga, Japan). The cDNAs were amplified using primers designed with ProbeFiber software (Roche Applied Science, Mannheim, Germany). Table 1 shows the primers used, the Roche Universal ProbeLibrary Probe numbers, and accession numbers. Amplification was performed with a LightCycler (Roche) using LightCycler TaqMan Master mix (Roche). The PCR reaction parameters were as follows : 95°C for 10 min, followed by 45 cycles (except for 18 s, for which 25 cycles were performed) of 95°C for 10 s, 60°C for 30 s, and 72°C for 1 s. Fluorescence data were analyzed with LightCycler software (Roche). The mRNA levels of target genes were compared with those of *18s* rRNA as a standard, and relative expression ratios were calculated[5].

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

Gene		Sequence	Accession number	Probe number
MMP-2	Sense primer	5'-gagggggcaggtcatgtag-3'	AY738117.1	#58
	Antisense primer	5'-ggctggatgagatcttgctg-3'		
MMP-7	Sense primer	5'-gctgacatcatgattggcttt-3'	NM_002423.3	#72
	Antisense primer	5'-tctcctccgagacctgtcc-3'		
MMP-9	Sense primer	5'-gaaccaatctcaccgacagg-3'	NM_004994.2	#6
	Antisense primer	5'-gccacccgagtgtaaccata-3'		
MMP-14	Sense primer	5'-ccctggatccccctacag-3'	AY795074.1	#56
	Antisense primer	5'-caaccaagaactggaaggaaa-3'		
TIMP-1	Sense primer	5'-cctccaacgggagtcctt-3'	AY932824.1	#73
	Antisense primer	5'-cagagctgcagagcaacaag-3'		
TIMP-2	Sense primer	5'-gaagagcctgaaccacaggt-3'	NM_003255.4	#43
	Antisense primer	5'-cggggaggagatgtagcac-3'		
TIMP-3	Sense primer	5'-gctggaggtcaacaagtacca-3'	NM_000362.4	#62
	Antisense primer	5'-cacagccccgtgtacatct-3'		
TIMP-4	Sense primer	5'ttggtgcagagggaaagtct-3'	NM_003256.3	#5
	Antisense primer	5'-ggtactgtgtagcaggtggtga-3'		
ITGA1	Sense primer	5'-aattggctctagtcaccattgtt-3'	NM_181501.1	#14
	Antisense primer	5'-caaatgaagctgctgactggt-3'		
ITGAV	Sense primer	5'-gccgtggatttcttcgtg-3'	NM_002210.3	#64
	Antisense primer	5'-gaggacctgccctccttc-3'		
ITGB5	Sense primer	5'-gggagtttgcaaagtttcagag-3'	NM_002213.3	#81
	Antisense primer	5'-tgtgcgtggagataggcttt-3'		
Periostin	Sense primer	5'-caccaaggtcaccaaattcat-3'	NM_001135934.1	#9
	Antisense primer	5'-ttcctcacgggtgtgtctc-3'		
18s	Sense primer	5'-gcaattattccccatgaacg-3'	X03205.1	#48
	Antisense primer	5'-gggacttaatcaacgcaagc-3'		

Statistical analysis

RTCA data were analyzed with the Bonferroni test following one-way analysis of variance (ANOVA)[5, 24]. RTCA data are expressed as the mean ± SD. Data for mRNA expression were examined using box plots and compared using the Kruskal–Wallis test. $P < 0.05$ was considered significant.

Results

The migratory response of head and neck cancer cell lines was evaluated using RTCA as an alternative non-invasive and label-free approach for continuous monitoring of cell migration on a cell culture level (Fig. 1A). The migratory response of TCO-1 cells increased quickly,

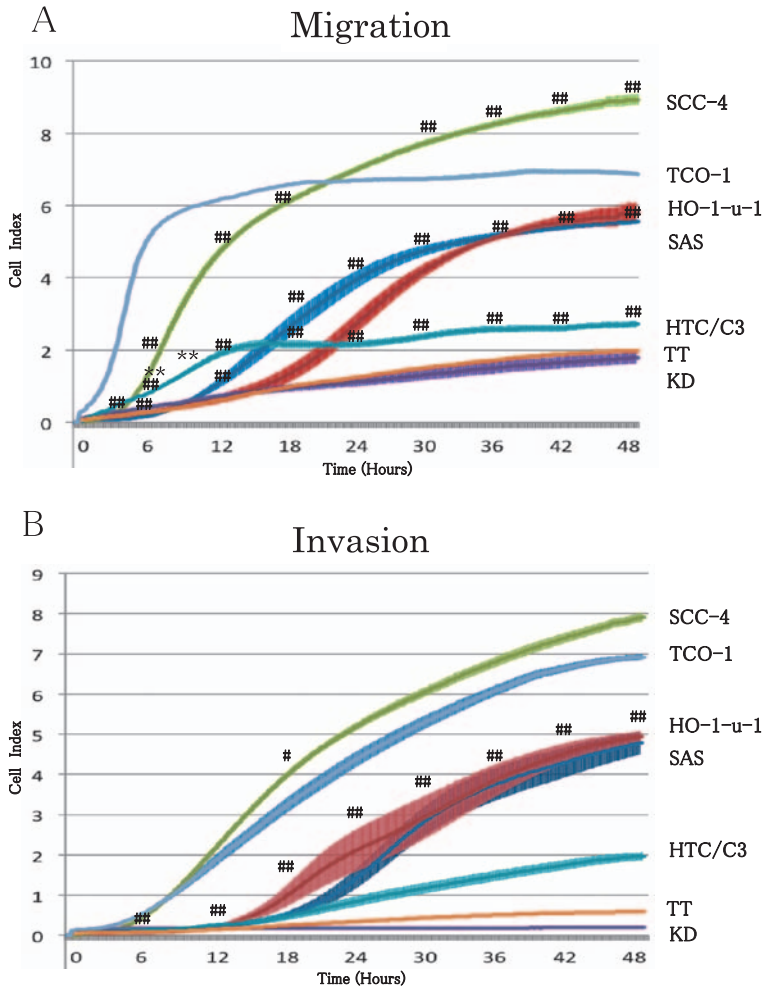

Fig. 1. (A) Migration and (B) invasion activity of HO-1-u-1 (red), SAS (blue), SCC-4 (green), TCO-1 (light blue), HTC / C3 (bluish green), T.T (orange), and KD (purple) cells as determined using real-time cell analysis (RTCA). Cells were resuspended in their respective growth medium and seeded into the wells of a CIM-Plate 16 for RTCA. Changes in impedance resulting from cells that had migrated to the bottom side of the membranes were recorded every 15 min and monitored over a total of 48 h. Data are the mean ± SD of seven to 12 wells. ** $P < 0.01$ compared with SAS cells; #$P < 0.05$, ##$P < 0.01$ compared with TCO-1 cells.

lasting for approximately 6 hours, at which point the cell index for TCO-1 cells was higher than that recorded for SAS and HO-1-u-1 cells. Although the migratory response of HTC / C3 cells increased quickly too, there was little growth in the increased migratory response of HTC / C3 cells. The migratory response of T.T and KD cells increased slightly and slowly over a period of 48 h (Fig. 1A).

The invasion responses of cells were similar to their migratory responses, except for TCO-1 and HTC / C3 cells, which clearly exhibited weaker and later invasion responses compared

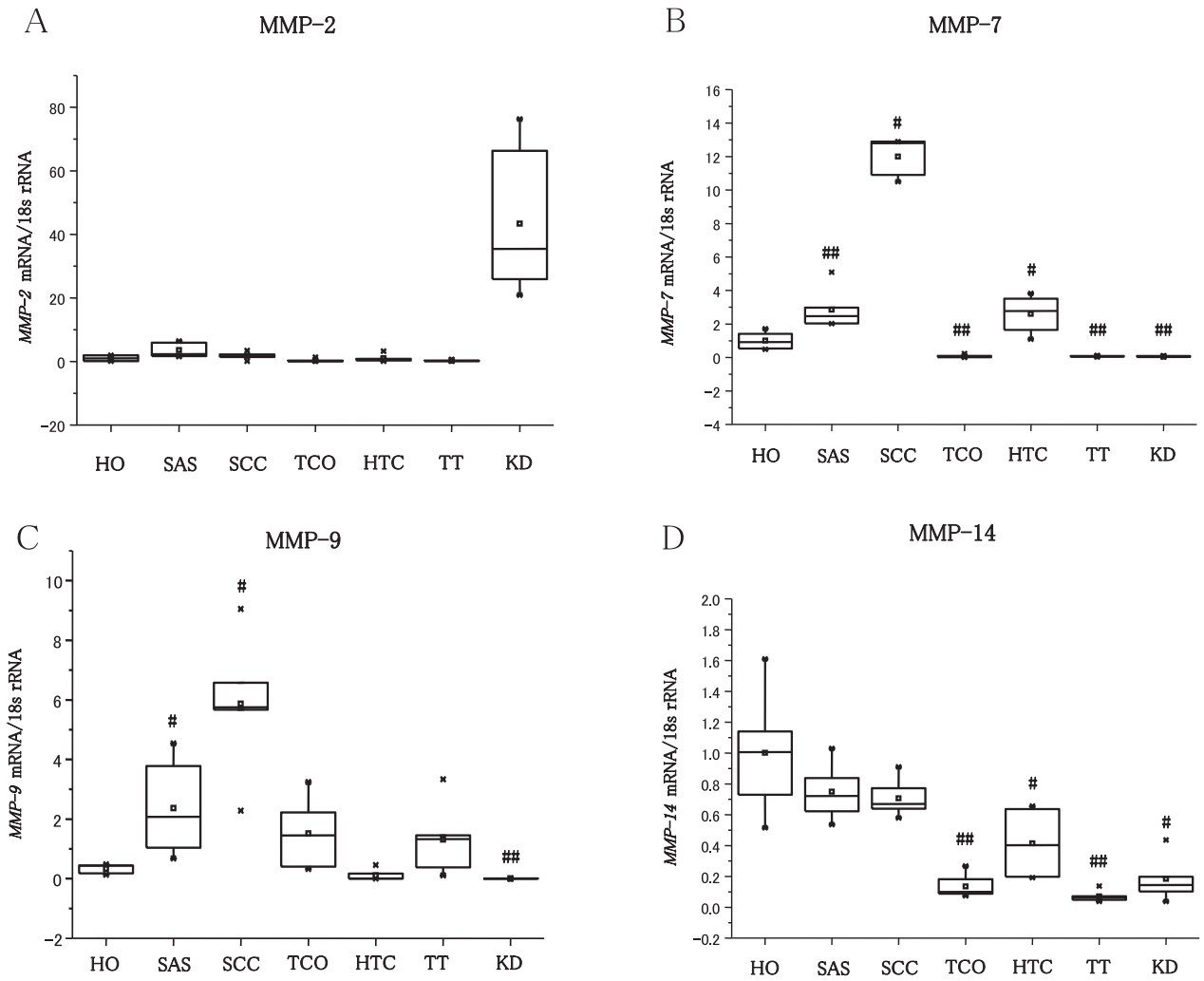

Fig. 2. (A) Matrix metalloproteinase-2 (*MMP-2*), (B) *MMP-7*, (C) *MMP-9*, and (D) *MMP-14* mRNA expression in HO-1-u-1, SAS, SCC-4, TCO-1, HTC / C3, T.T, and KD cells relative to HO-1-u-1 cells, as determined using quantitative real-time polymerase chain reaction (n = 3-6). Boxes indicate the interquartile range, horizontal lines indicate median values, and whiskers indicate minimum and maximum values. #$P < 0.05$, ##$P < 0.01$ compared with HO-1-u-1 cells.

with migratory responses (Fig. 1B). The order of the migratory activity of cells as determined by RTCA was TCO-1 > SCC-4 > SAS > HO-1-u-1 > HTC / C3 > T.T = KD cells. However, the order of the invasion activity, as determined by RTCA, was SCC-4 = TCO-1 > SAS = HO-1-u-1 > HTC / C3 > T.T > KD cells.

Expression of *MMP*, *TIMP*, *ITG*, and *periostin* mRNAs is shown in Figs. 2–5, respectively. The level of each mRNA in HO-1-u-1 cells was set at 100%. In KD cells, *MMP-2* mRNA expression was 36.74-fold greater than that in HO-1-u-1 cells ($P = 0.067$; Fig. 2A). In SAS, SCC-4, and HTC / C3 cells, *MMP-7* mRNA levels were 2.67-fold ($P = 0.0081$), 13.81-fold ($P = 0.012$), and 3.00-fold ($P = 0.036$) higher than in HO-1-u-1 cells, respectively (Fig. 2B). In contrast, *MMP-7* mRNA levels were very low in TCO-1, T.T, and KD cells. *MMP-9* mRNA

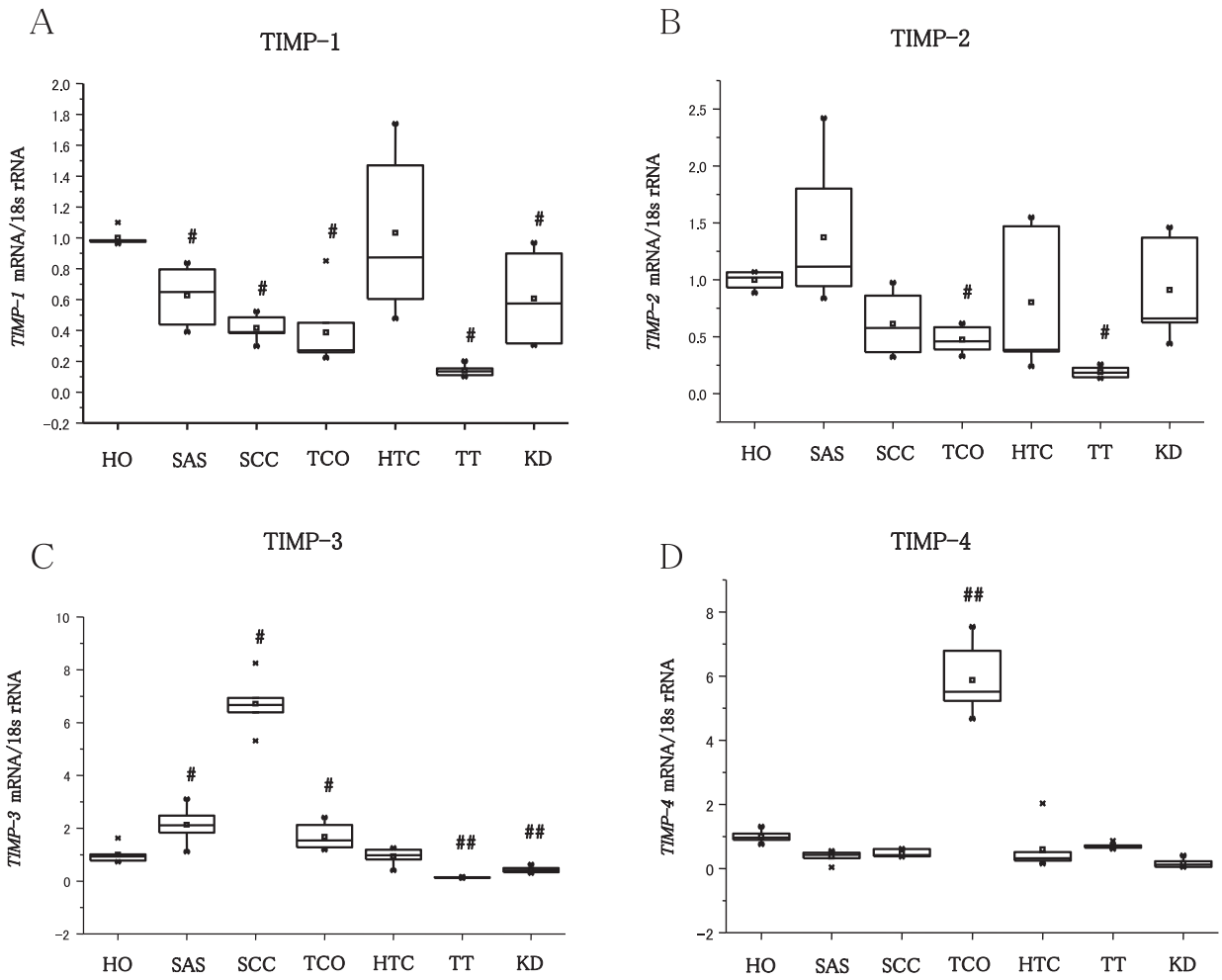

Fig. 3. (A) Tissue-specific inhibitor of metalloproteinase-1 (*TIMP-1*), (B) *TIMP-2*, (C) *TIMP-3*, and (D) *TIMP-4* mRNA expression in HO-1-u-1, SAS, SCC-4, TCO-1, HTC / C3, T.T, and KD cells relative to HO-1-u-1 cells, as determined using quantitative real-time polymerase chain reaction (n = 4-6). Boxes indicate the interquartile range, horizontal lines indicate median values, and whiskers indicate minimum and maximum values. #$P < 0.05$, ##$P < 0.01$ compared with HO-1-u-1 cells.

levels in SCC-4 cells were 13.16-fold ($P = 0.020$) and 3.96-fold ($P = 0.014$) higher than in HO-1-u-1 and TCO-1 cells, respectively (Fig. 2C). In contrast, *MMP-9* mRNA expression was not detected in HTC / C3 and KD cells. *MMP-14* mRNA levels in HTC / C3, TCO-1, T.T, and KD cells were 0.40-fold ($P = 0.023$), 0.10-fold ($P = 0.0081$), 0.062-fold ($P = 0.037$), and 0.14-fold ($P = 0.012$) lower than in HO-1-u-1 cells, respectively (Fig. 2D). *TIMP-1* and *TIMP-2* mRNA levels in T.T cells were 0.14-fold ($P = 0.014$) and 0.18-fold ($P = 0.037$) higher than in HO-1-u-1 cells, respectively (Fig. 3A, B), whereas *TIMP-3* mRNA levels in SAS and SCC-4 cells were 2.27-fold ($P = 0.014$) and 7.16-fold ($P = 0.012$) higher than in HO-1-u-1 cells, respectively (Fig. 3C). *TIMP-3* mRNA levels in T.T and KD cells were 0.14-fold ($P = 0.0081$) and 0.44-fold ($P = 0.0081$) lower than in HO-1-u-1 cells, respectively (Fig. 3C). *TIMP-4* mRNA levels in TCO-1 cells were 5.70-fold ($P = 0.0081$) higher than in HO-1-u-1 cells (Fig. 3D).

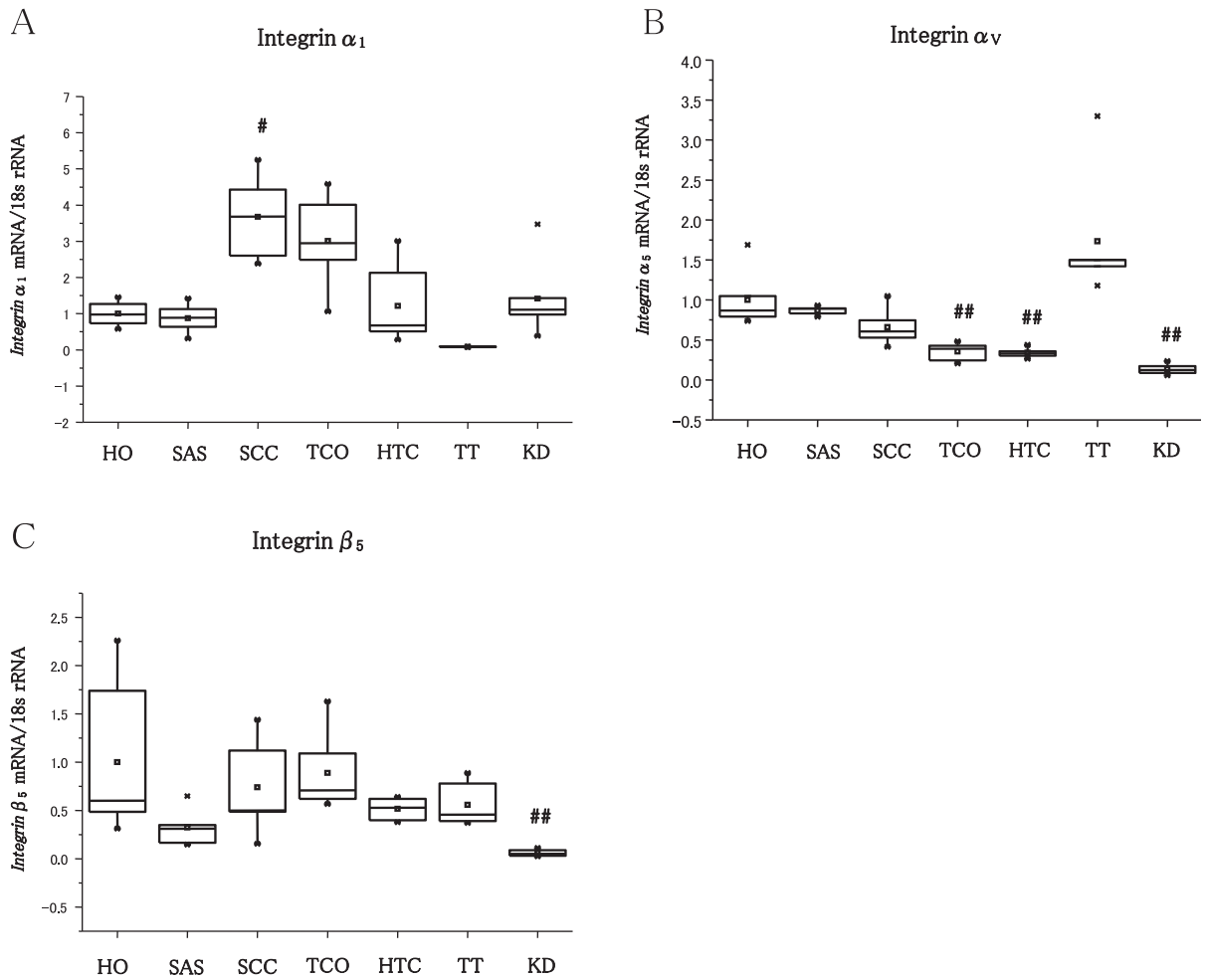

Fig. 4. (A) *ITGA1*, (B) *ITGAV*, and (C) *ITGB5* mRNA expression in HO-1-u-1, SAS, SCC-4, TCO-1, HTC / C3, T.T, and KD cells relative to HO-1-u-1 cells, as determined using quantitative real-time polymerase chain reaction (n = 5-6). Boxes indicate the interquartile range, horizontal lines indicate median values, and whiskers indicate minimum and maximum values. #$P < 0.05$, ##$P < 0.01$ compared with HO-1-u-1 cells.

Compared with levels in HO-1-u-1 cells, *ITGA1* mRNA levels were 3.75-fold ($P = 0.028$) and 3.01-fold ($P = 0.053$) higher in SCC-4 and TCO-1 cells, respectively (Fig. 4A), and 0.09-fold ($P = 0.052$) lower in T.T cells. Conversely, *ITGAV* mRNA levels were 1.73-fold ($P = 0.080$) higher in T.T than HO-1-u-1 cells (Fig. 4B), and 0.14-fold ($P = 0.0081$) lower in KD than HO-1-u-1 cells. Compared with HO-1-u-1 cells, *ITGB5* mRNA levels were 0.52-fold ($P = 0.075$) and 0.086-fold ($P = 0.0081$) lower in SAS and KD cells, respectively (Fig. 4C). Periostin mRNA levels were markedly higher in TCO-1 than in HO-1-u-1 cells (563-fold ; $P = 0.0081$; Fig. 5).

Discussion

In the present study, using RTCA we demonstrated that TCO-1 cells (ATC) had very strong migratory responses compared with OSCC cell lines, and also exhibited a marked increase in the

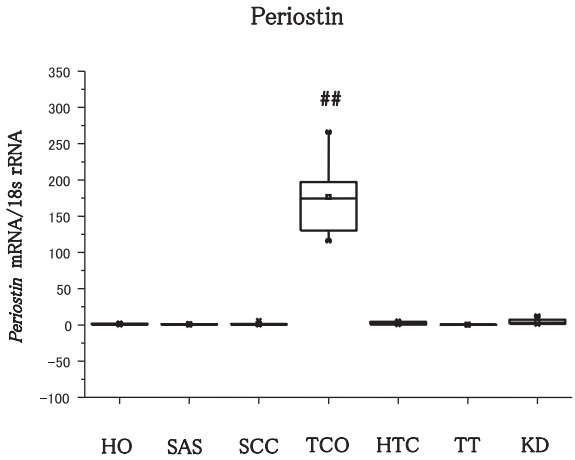

Fig. 5. Periostin mRNA expression in HO-1-u-1, SAS, SCC-4, TCO-1, HTC/C3, T.T, and KD cells relative to HO-1-u-1 cells, as determined using quantitative real-time polymerase chain reaction (n = 5-6). Boxes indicate the interquartile range, horizontal lines indicate median values, and whiskers indicate minimum and maximum values. ##$P < 0.01$ compared with HO-1-u-1 cells.

expression of periostin mRNA. Using RTCA, we were able to capture the characteristics of the different cell lines.

ATC is one of the most malignant human cancers. At the time of diagnosis, 90% of patients with ATC present with unresectable locally advanced or distant metastatic disease. Therefore, the median survival of patients with ATC is less than 6 months[3]. One of the causes of the high malignancy of ATC is the high migratory activity, as demonstrated by RTCA. We believe that one of the factors supporting the high migratory activity of TCO-1 cells is periostin. Gillan *et al* reported that periostin, by promoting $\alpha_V\beta_3$ or $\alpha_V\beta_5$ integrin-dependent adhesion and migration of ovarian epithelial cells, it promotes i.p. dissemination[18]. In the present study, there was no increase in α_V integrin and β_5 integrin mRNA expression in TCO-1 compared with OSCC cell lines. However, in the present study, periostin to support integrins expressed too much. In fact, Kyutoku *et al* reported inhibition of breast cancer progression and metastasis by an anti-periostin antibody in a murine model[25]. In recent years, increasing numbers of single center studies have reported periostin overexpression in various human gastrointestinal carcinoma types as an independent prognostic factor for predicting decreased overall survival rates following tumor resection[19]. In the present study, the increase in α_1 integrin in TCO-1 cells was almost the same as that in SCC-4 cells. It is fasciclin I, not periostin, that interacts with α_1 integrin, because periostin does not contain an RGD motif[18].

MMPs and TIMPs play crucial roles in tumor cell invasion and metastasis. MMP-2 expression is known to be greater in fibroblasts than in cancer cells and it is constitutively expressed in fibroblasts like a housekeeping gene[26]. In the present study, *MMP-2* mRNA expression was

particularly high in the KD cell line, from human fibroblasts of the lip. TIMPs tightly control MMP activity in a 1 : 1 ratio[27]. However, MMP-14 associates with pro-MMP-2 and TIMP-2 at the cell surface and regulates MMP-2 activation[15]. In the present study, mRNA expression of *MMP-14* and *TIMP-2* was not increased in KD cells compared with other the other cell lines, thus indicating that there is not much activated MMP-2 present in KD cells compared with other cells. MMP-7 (matrilysin) is the smallest member of the MMP family and is primarily expressed by mucosal tissue[28]. In the present study, SCC-4 cells exhibited a marked increase in *MMP-7* mRNA expression. Ahmed *et al* reported MMP-7 expression primarily in the invasive portion of the tumor in the case of both oral and cutaneous cancers[28]. Interestingly, although TCO-1 cells exhibited invasive activity similar to that of SCC-4 cells, *MMP-7* and *MMP-9* mRNA expression was considerably lower in TCO-1 than in SSC-4 cells. TIMP-3 has the capacity to associate with both pro-MMP-2 and pro-MMP-9[29]. In the case of all cells, the mRNA expression of *TIMP-3* was very similar to that of *MMP-9*. High TIMP-4 expression has been found to be correlated with tumor aggressiveness and to stimulate tumorigenic activity in several cancers[29]. In the present study, *TIMP-4* mRNA expression was very high in TCO-1 cells (ATC) compared with other cells.

In the present study we used RTCA technology to show the migratory and invasive responses associated with periostin, integrins, MMPs, and TIMPs in various head and neck cell lines in real time. We were able to identify the features of each of the cell lines, particularly TCO-1 cells. The results of the present study show that TCO-1 cells have high migratory and invasive activity, and high levels of periostin and TIMP-4 expression. We suggest that TIMP-4 and periostin, in particular, may be potential targets and biomarkers for the treatment of ATC.

Conflict of interest

The authors declare they have no potential conflicts of interest.

References

1) Ranganath R, Shah MA, Shah AR. Anaplastic thyroid cancer. *Curr Opin Endocrinol Diabetes Obes*. 2015;**22**:387–391.
2) Shaha AR. Anaplastic thyroid cancer: large database, cautious interpretations. *Ann Surg Oncol*. 2015;**22**:4113–4114.
3) Sadowski SM, Boufraqech M, Zhang L, *et al*. Torin2 targets dysregulated pathways in anaplastic thyroid cancer and inhibits tumor growth and metastasis. *Oncotarget*. 2015;**6**:18038–18049.
4) Thiery JP. Epithelial–mesenchymal transitions in tumour progression. *Nat Rev Cancer*. 2002;**2**:442–454.
5) Ono T, Iwai S, Egawa S, *et al*. Investigation of cell migration and invasion using real-time cell analysis, as well as the association with matrix metalloproteinase-9 in oral squamous cell carcinomas. *Showa Univ J Med Sci*. 2013;**25**:203–212.
6) Atienza JM, Yu N, Kirstein SL, *et al*. Dynamic and label-free cell-based assays using the real-time cell electronic sensing system. *Assay Drug Dev Technol*. 2006;**4**:597–607.
7) Uemura S, Matsushita H, Li W, *et al*. Diabetes mellitus enhances vascular matrix metalloproteinase activity: role of oxidative stress. *Circ Res*. 2001;**88**:1291–1298.
8) Visse R, Nagase H. Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. *Circ Res*. 2003;**92**:827–839.

9) Nagase H, Woessner JF Jr. Matrix metalloproteinases. *J Biol Chem*. 1999;**274**:21491-21494.
10) Komatsu K, Nakanishi Y, Nemoto N, *et al*. Expression and quantitative analysis of matrix metalloproteinase-2 and -9 in human gliomas. *Brain Tumor Pathol*. 2004;**21**:105-112.
11) Chambers AF, Matrisian LM. Changing views of the role of matrix metalloproteinases in metastasis. *J Natl Cancer Inst*. 1997;**89**:1260-1270.
12) Yu T, Wu Y, Helman JI, *et al*. CXCR4 promotes oral squamous cell carcinoma migration and invasion through inducing expression of MMP-9 and MMP-13 via the ERK signaling pathway. *Mol Cancer Res*. 2011;**9**:161-172.
13) Chen ST, Liu DW, Lin JD, *et al*. Down-regulation of matrix metalloproteinase-7 inhibits metastasis of human anaplastic thyroid cancer cell line. *Clin Exp Metastasis*. 2012;**29**:71-82.
14) Arpino V, Brock M, Gill SE. The role of TIMPs in regulation of extracellular matrix proteolysis. *Matrix Biol*. 2015;**44-46**:247-254.
15) Vincenti MP. The matrix metalloproteinase (MMP) and tissue inhibitor of metalloproteinase (TIMP) genes. In *Clark IM ed*. *Matrix metalloproteinase protocols*. Totowa: Humana Press; 2001. pp 121-148. (Methods in molecular biology; 151).
16) Iwamoto DV, Calderwood DA. Regulation of integrin-mediated adhesions. *Curr Opin Cell Biol*. 2015;**36**:41-47.
17) Daley WP, Peters SB, Larsen M. Extracellular matrix dynamics in development and regenerative medicine. *J Cell Sci*. 2008;**121**:255-264.
18) Gillan L, Matei D, Fishman DA, *et al*. Periostin secreted by epithelial ovarian carcinoma is a ligand for alpha(V) beta(3) and alpha(V) beta(5) integrins and promotes cell motility. *Cancer Res*. 2002;**62**:5358-5364.
19) Sirica AE, Almenara JA, Li C. Periostin in intrahepatic cholangiocarcinoma: pathobiological insights and clinical implications. *Exp Mol Pathol*. 2014;**97**:515-524.
20) Higashi T, Sasai H, Suzuki F, *et al*. Hamster cell line suitable for transfection assay of transforming genes. *Proc Natl Acad Sci U S A*. 1990;**87**:2409-2413.
21) Enomoto T, Sugawa H, Inoue D, *et al*. Establishment of a human undifferentiated thyroid cancer cell line producing several growth factors and cytokines. *Cancer*. 1990;**65**:1971-1979.
22) Leavitt J, Bushar G, Kakunaga T, *et al*. Variations in expression of mutant beta actin accompanying incremental increases in human fibroblast tumorigenicity. *Cell*. 1982;**28**:259-268.
23) Limame R, Wouters A, Pauwels B, *et al*. Comparative analysis of dynamic cell viability, migration and invasion assessments by novel real-time technology and classic endpoint assays. *PLoS One* (Internet). 2012;**7**:e46536. (accessed 2016 Feb 12) Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0046536
24) Mahmood A, Pulakat L. Differential effects of β-blockers, angiotensin II receptor blockers, and a novel AT2R agonist NP-6A4 on stress response of nutrient-starved cardiovascular cells. *PLoS One* (Internet). 2015;**10**:e0144824. (accessed 2016 Feb 12) Available from: http://journals.plos.org/plosone/article?id=10.1371/journal.pone.0144824
25) Kyutoku M, Taniyama Y, Katsuragi N, *et al*. Role of periostin in cancer progression and metastasis: inhibition of breast cancer progression and metastasis by anti-periostin antibody in a murine model. *Int J Mol Med*. 2011;**28**:181-186.
26) Kobayashi S, Iwai S, Tsujiyama K, *et al*. Theaflavin-3,3′-digallate inhibits tube formation in cocultured endothelial cells with fibroblasts. *Showa Univ J Med Sci*. 2007;**19**:59-72.
27) Cawston TE, Wilson AJ. Understanding the role of tissue degrading enzymes and their inhibitors in development and disease. *Best Pract Res Clin Rheumatol*. 2006;**20**:983-1002.
28) Ahmed HOA, Haglund C, Virolainen S, *et al*. MMP-7, MMP-8, and MMP-9 in oral and cutaneous squamous cell carcinomas. *Oral Surg Oral Med Oral Pathol Oral Radiol*. 2015;**119**:459-467.
29) Rorive S, Lopez XM, Maris C, *et al*. TIMP-4 and CD63: new prognostic biomarkers in human astrocytomas. *Mod Pathol*. 2010;**23**:1418-1428.

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