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Dermatopontin: a potential predictor for metastasis of human oral cancer

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Dermatopontin (DPT), a component of the extracellular matrix (ECM), is involved in promotion of cellular adhesion and ECM assembly activities. However, the role of DPT in the pathogenesis of carcinoma is unclear. We evaluated DPT expression in human oral cancer and its possible roles including cellular adhesion and invasiveness. We first investigated the DPT mRNA and protein expression status in human oral squamous cell carcinoma (OSCC)-derived cells. Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) and immunoblotting analysis detected frequent downregulation of DPT in OSCC-derived cells compared to human normal oral keratinocytes. To assess the epigenetic regulation of DPT, OSCC-derived cells were treated with a histone deacetylase inhibitor, sodium butyrate (NaB). NaB restored the DPT expression in OSCC-derived cells. DPT-overexpressed cells were examined whether DPT could contribute to cellular adhesion and invasiveness. Markedly, increased adhesion and decreased invasiveness in DPT-overexpressed cells were found compared to mock-transfected cells. Adhesion of DPT-overexpressed cells was inhibited by α3β1 integrin functional blocking antibody. OSCC-derived cells treated with NaB also decreased invasiveness. The expression status of DPT in primary OSCCs ($n = 97$) was analyzed and compared to clinicopathological behavior. DPT expression in primary OSCCs was significantly lower ($p < 0.05$) than in the normal counterparts and was correlated significantly ($p < 0.05$) with regional lymph node metastasis. Our data provided strong evidence that downregulation of DPT is a characteristic event in OSCCs and that DPT was correlated with cellular adhesion and invasiveness. Therefore, DPT might play an important role in regulating tumor invasion and metastasis.

Tumor invasion and metastasis associated with neoplastic progression are major causes of cancer deaths, including oral squamous cell carcinoma (OSCC).[1] Improvements of prognosis in OSCC depend on the elucidation of the biologic and molecular mechanisms underlying metastatic diseases. In this context, the extracellular matrix (ECM) has been shown to play a pivotal role in metastasis.[2] The invasiveness and metastatic processes occur through interactions between the cancer cells and the ECM, which is a complex structural entity that surrounds and supports the cells within living systems.[3] In vertebrates, the ECM consists of collagens[4,5] and noncollagenous proteins, such as proteoglycans.[6] The functions of the noncollagenous proteins are not limited only to the maintenance of tissue integrity and architecture, but many of them also affect cellular behavior.[7] Among the noncollagenous proteins of the ECM, the small leucine-rich proteoglycans are some of the best-characterized molecules. One such proteoglycan, decorin, is involved in the formation of tissue structure by affecting fibrillogenesis.[8] Decorin binds to the epidermal growth factor receptor, which leads to a protracted downregulation of its activity.[9,10]

Dermatopontin (DPT), a noncollagenous ECM protein, is initially copurifed as a 22 kDa protein from bovine dermal extracts during the course of decorin purification.[11] DPT is acidic and rich in tyrosine residues[12] and was indicated that about half of tyrosine residues are sulfated.[13] Similar sulfated tyrosine residues also have been seen in other ECM proteins,[14–16] and sulfated tyrosine is important for interacting with other ECM proteins.[17,18] The richest source of DPT appears to be skin, in which DPT is estimated to comprise 15 mg/kg of the wet weight.[19] DPT comprises a considerable proportion of the noncollagenous ECM proteins. However, the functional characterization of DPT has not been fully elucidated. Recently, DPT has been shown to be a multifunctional adhesion molecule for epidermal cells.[20]

Key words: dermatopontin, oral squamous cell carcinoma, adhesion, invasiveness, metastasis

Abbreviations: DPT, dermatopontin; ECM, extracellular matrix; HNOKs, human normal oral keratinocytes; IHC, immunohistochemistry; NaB, sodium butyrate; OSCC, oral squamous cell carcinoma; qRT-PCR, real-time quantitative reverse transcriptase-polymerase chain reaction.

Additional Supporting Information may be found in the online version of this article.

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In the present study, we evaluated the expression status of DPT and the effect in OSCC-derived cells overexpressing DPT on cellular adhesion and invasiveness, which are one of the vital aspects of metastases. In addition to our previous microarray analyses that showed that DPT was one of the downregulated genes in the OSCC-derived cells,[21] we found that frequent downregulation of DPT was observed in primary OSCCs and that DPT transfectants showed disturbance of adhesion and invasive activities through a mechanism mediated by integrin. The current study shows the results of a comprehensive analysis of molecular subtypes of DPT in OSCC that are clinically and functionally linked to metastasis.

Materials and Methods

Cells

HSC-2, HSC-3, HSC-4 and Ca9-22, derived from human OSCCs, were purchased from the Human Science Research Resources Bank (Osaka, Japan). H1 and Sa3 were kindly provided by Dr. S. Fujita at Wakayama Medical University (Wakayama, Japan). Primary cultured human normal oral keratinocytes (HNOKs) were obtained from three healthy donors and were used as a normal control.[21] All cell lines were maintained at 37°C (humidified atmosphere 5% CO_2/95% air) and cultured in Dulbecco's modified Eagle's medium (DMEM) F-12 HAM (Sigma Aldrich, St., MO) with 10% fetal bovine serum (FBS; Sigma) and 50 U/ml of penicillin and streptomycin (Sigma).

Tissue specimens

Ninety-seven pairs of primary OSCC samples and corresponding normal oral epithelial tissues were obtained at the time of surgeries performed at Chiba University Hospital. All patients provided informed consent for use of the protocol reviewed and approved by the institutional review board of Chiba University. The tissues were divided into two parts, one of which was frozen immediately and stored at −80°C until RNA isolation, and the second part was fixed in 10% buffered formaldehyde solution for pathologic diagnosis and immunohistochemistry (IHC). Histopathologic diagnostic procedures were performed according to the World Health Organization criteria by the Department of Pathology, Chiba University Hospital. Clinicopathologic staging was determined by the TNM classification of the International Union against Cancer. All OSCC samples were confirmed histologically and checked to ensure the presence of tumor in greater than 80% of specimens.

Evaluation of DPT mRNA expression

Total RNA was isolated from cells and tissues using Trizol Reagent (Invitrogen, Carlsbad, CA) and reverse-transcribed by Ready-to-Go You-Prime first-strand beads (GE Healthcare, Buckinghamshire, UK) and Oligo (dT) primer (Invitrogen). Real-time quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR) was performed to evaluate the *DPT* mRNA expression using a LightCycler FastStart DNA Master SYBR Green 1 Kit (Roche Diagnostics GmbH, Mannheim, Germany). The primer sequences for *DPT* mRNA expression were forward 5′-CCTACACGAAGCACCAGACA-3′ and reverse 5′-GATCCGGTTTTCTCGATTCA-3′. Amplified products were analyzed by 3% agarose gel electrophoresis to ascertain size and purity. The PCR reactions using LightCycler apparatus (Roche) were carried out in a final volume of 20 μl of a reaction mixture comprised of 2 μl of FirstStart DNA Master SYBR Green I mix, 3 mM $MgCl_2$ and 1 μM primers, according to the manufacturer's instructions. The reaction mixture was then loaded into glass capillary tubes and subjected to an initial denaturation at 95°C for 10 min, followed by 35 rounds of amplification at 95°C (10 sec) for denaturation, 62°C (5 sec) for annealing and 72°C (8 sec) for extension, with a temperature slope of 20°C/sec. The transcript amount of the *DPT* gene was estimated from the respective standard curves and normalized to the *glyceraldehyde-3-phosphate dehydrogenase* (forward 5′-CATCTCTGCCC CCTCTGCTGA-3′ and reverse 5′-GGATGACCTTGCCCA CAGCCT-3′) transcript amount determined in corresponding samples.

Protein extraction

The cells were washed twice with cold phosphate-buffered saline (PBS) and centrifuged at 1,000*g*. Cytoplasmic fractions from cultured cells were prepared using the NE-PER Nuclear and Cytoplasmic Extraction Reagents (Thermo, Rockford, IL). The protein concentration was measured by BCA protein assay (Thermo).

Immunoblotting analysis

Cytoplasmic proteins (50 μg) were separated by SDS-PAGE in 4–12% gel, transferred to nitrocellulose membranes and blocked for 1 hr at room temperature in Blocking One (Nacalai Tesque, Tokyo, Japan). A 22.8 kDa recombinant human DPT (R&D Systems, Minneapolis, MN) was used as positive control. The membrane was incubated with antibodies against DPT (Proteintech Group, Chicago, IL) for 4 hr at room temperature and β-actin for 1 hr at room temperature. The membrane was washed with 0.1% Tween-20 in Tris-buffered saline, incubated with secondary antibodies and coupled to horseradish peroxidase-conjugated anti-rabbit or anti-mouse IgG for 1 hr at room temperature. The proteins were detected by SuperSignal Chemiluminescent substrate (Thermo). Finally, the results of immunoblotting analysis were visualized by exposing the membrane to a cooled CCD camera system, Light Capture (ATTO, Tokyo, Japan). Signal intensities were quantitated using CS Analyzer version 3.0 (ATTO).

Treatment with an HDAC inhibitor

OSCC-derived cells were treated with a histone deacetylase (HDAC) inhibitor, sodium butyrate (NaB) (Wako, Osaka,

Japan). All OSCC-derived cells were plated at 50% confluence and treated with 2.5 or 5.0 mM NaB for 12 or 24 hr.

Transfection of DPT plasmid

OSCC-derived cells (H1 and Sa3) were transfected with DPT plasmid designed to overexpress DPT cDNA (Santa Cruz Biotechnology, Santa Cruz, CA) using Lipofectamine LTX (Invitrogen). After transfection, the cells that stably overexpressed DPT were isolated by Neomycin (Invitrogen). Two to three weeks after transfection, viable colonies were transferred to new dishes.

Cellular growth

To evaluate the effect of DPT overexpression on cellular proliferation, we first analyzed cellular growth in DPT- and empty vector (mock)-transfected cells. The experiments were carried out for 7 days by counting the cells every 24 hr. At the indicated time point, the cells were trypsinized and counted using a haemocytometer in triplicate samples. We compared the number of the DPT- and mock-transfected cells.

Adhesion assay

The DPT- and mock-transfected cells were grown in DMEM F-12 HAM with 10% FBS until reaching 80% confluence. The cells were then washed three times with PBS, treated with 0.25% trypsin-EDTA solution (Sigma) and collected in the serum-containing medium. After centrifugation, supernatant was removed, DMEM F-12 HAM (serum-free) was added to the cell pellet and the cells were resuspended. A total of 4×10^5 cells were seeded to each dish and incubated at 37°C for 2 hr. The dishes were then rinsed with PBS. The adherent cells were incubated at 37°C for 72 hr in DMEM F-12 HAM with 10% FBS. The cells were stained with crystal violet/methanol. Five minutes later, the dishes were washed with water, dried completely and the number of cells on each dish was counted.

Inhibition of cell adhesion by blocking α3β1 integrin

The DPT- and mock-transfected cells were harvested and resuspended in the serum-free medium. The cell suspension was incubated with α3β1 integrin monoclonal antibody (Millipore, Billerica, MA; final concentration 50 μg/ml) for 30 min at room temperature. The cells were then seeded at a density of 4×10^5 cells/dish and incubated at 37°C for 2 hr. The dishes were then rinsed with PBS. The adherent cells were incubated at 37°C for 72 hr in DMEM F-12 HAM with 10% FBS. The cells were stained with crystal violet/methanol, and the number of cells was counted.

Invasiveness assay

A total of 2.5×10^5 cells resuspended in the serum-free medium were seeded on a polyethylene terephthalate membrane insert with a pore size of 3 μm in a transwell apparatus (Becton Dickinson Labware, Franklin Lakes, NJ). In the lower chamber, 2 ml of serum-free medium was added. After the cells were incubated for 22 hr at 37°C, the inset was washed with PBS, and the cells on the top surface of the insert were removed with a cotton swab. Cells adhering to the lower surface of the membrane were stained with crystal violet/methanol, and the number of cells entering the pores in five random fields was counted using a light microscope at ×100 magnification.

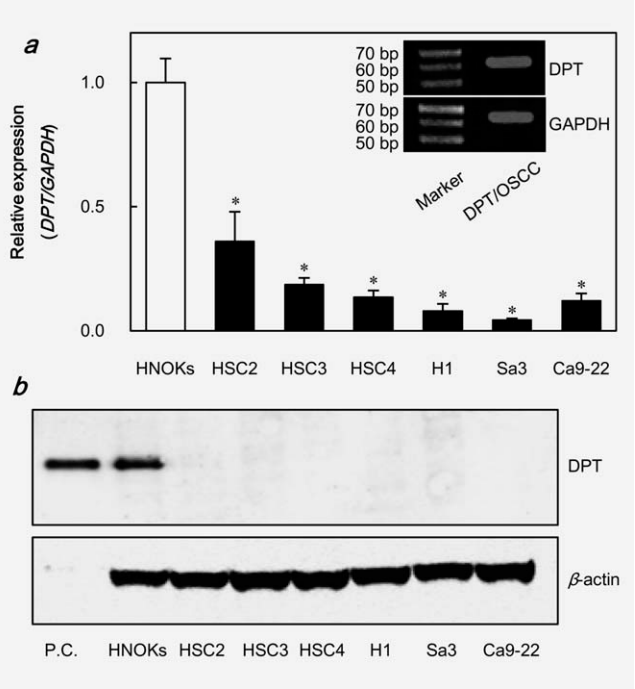

Figure 1. Evaluation of DPT expression in OSCC-derived cell lines. (*a*) *DPT* mRNA levels were analyzed in OSCC-derived cells and HNOKs by qRT-PCR analysis. Significant downregulation of *DPT* mRNA was observed in six OSCC-derived cells compared with that in HNOKs (*$p < 0.05$, Mann–Whitney's *U* test). Data are expressed as the mean ± SEM in triplicate. The PCR product is electrophoresed on 3% agarose gel and visualized using ethidium bromide stain. The band appears as the appropriate size. (*b*) Representative immunoblotting analysis of DPT protein in OSCC-derived cells and HNOKs. DPT protein expression was downregulated in all OSCC-derived cells compared to HNOKs. (P.C., positive control).

IHC

Immunohistochemistry (IHC) of 4 μm sections of paraffin-embedded specimens was performed using rabbit anti-DPT polyclonal antibody. Briefly, after deparaffinization and hydration, the endogenous peroxidase activity was quenched by 30 min incubation in a mixture of 0.3% hydrogen peroxide solution in 100% methanol, after which the sections were blocked for 2 hr at room temperature with 1.5% blocking serum (Santa Cruz Biotechnology) in PBS before reaction with anti-DPT antibody (1:100 dilution) at 4°C in a moist chamber overnight. Upon incubation with the primary antibody, the specimens were washed three times in PBS and treated with Envision reagent (DAKO, Carpinteria, CA) followed by color development in 3,3′-diaminobenzidine tetrahydrochloride (DAKO). The slides

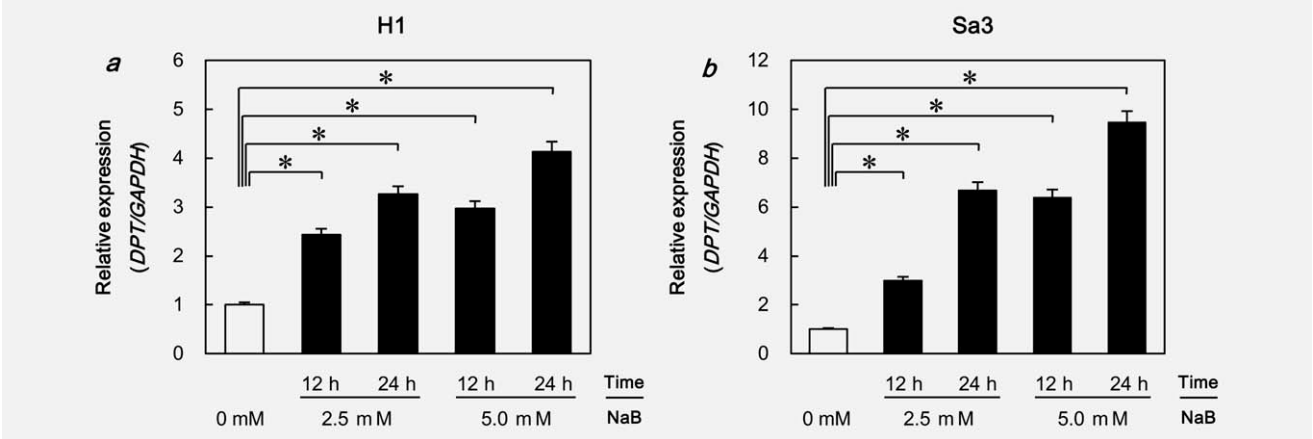

Figure 2. The effect of NaB on *DPT* mRNA expression in OSCC-derived cells. (*a*) H1 and (*b*) Sa3 cells were treated with 2.5 or 5.0 mM NaB for 12 or 24 hr. *DPT* mRNA levels were restored in NaB-treated cells compared to untreated cells (*$p < 0.05$, Mann–Whitney's *U* test). Data are expressed as the mean ± SEM in triplicate.

then were counterstained lightly with hematoxylin, dehydrated with ethanol, cleaned with xylene and mounted. Nonspecific binding of an antibody to proteins other than the antigen sometimes occurred; to avoid this, an immunizing peptide blocking experiment was performed. As a negative control, triplicate sections were immunostained without exposure to primary antibodies, which confirmed the staining specificity. To quantify the state of DPT protein expression in those components, we used the IHC score system described previously.[22] This IHC score system was established for semiquantitative evaluation of IHC

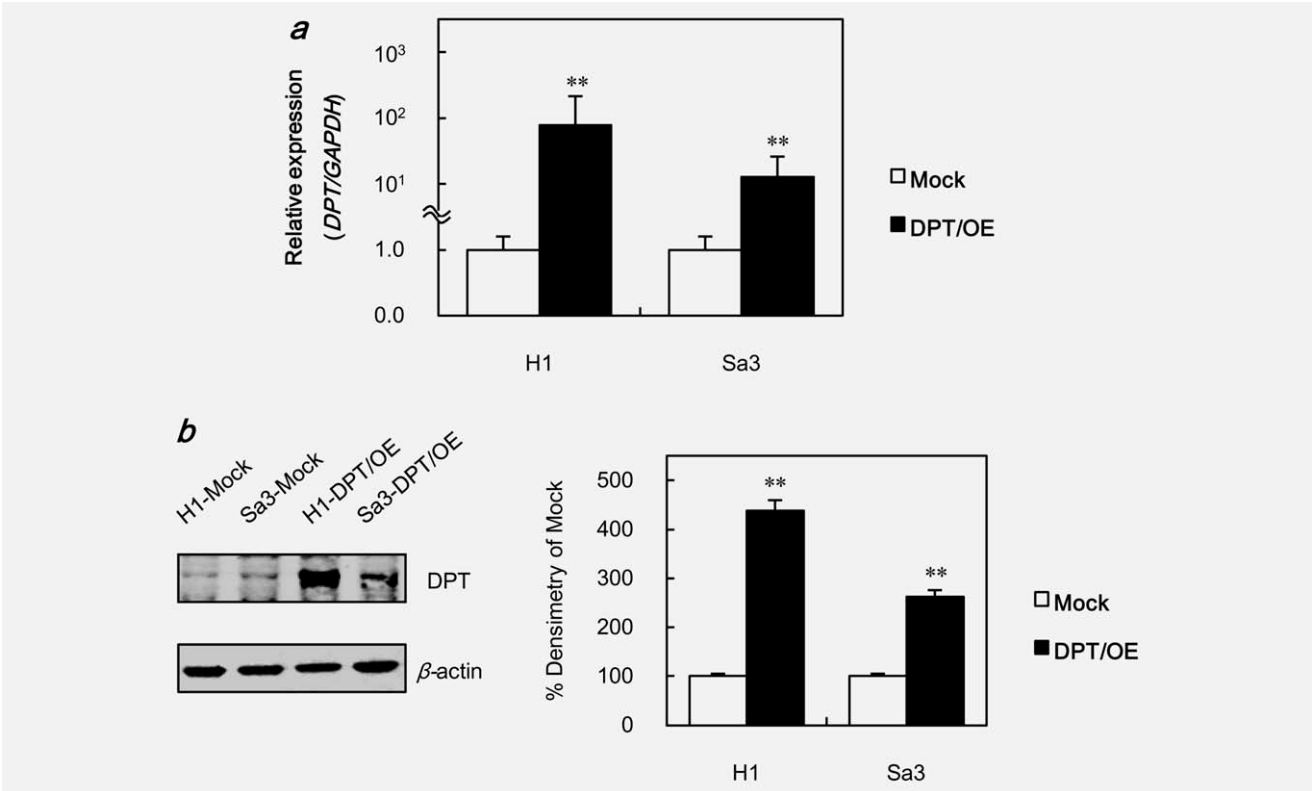

Figure 3. mRNA and protein expression in DPT-transfected (H1 and Sa3) cells using qRT-PCR and immunoblotting analyses. (*a*) *DPT* mRNA levels in DPT-transfected cells. qRT-PCR showed that *DPT* was overexpressed in DPT-transfected cells compared with mock-transfected cells (**$p < 0.001$, Mann–Whitney's *U* test). Data are expressed as the mean ± SEM of triplicate results. (*b*) Representative immunoblotting analysis and densitometric data of DPT protein levels in DPT- and mock-transfected cells. DPT protein markedly increased in DPT-transfected cells compared mock-transfected cells (**$p < 0.001$, Mann–Whitney's *U* test).

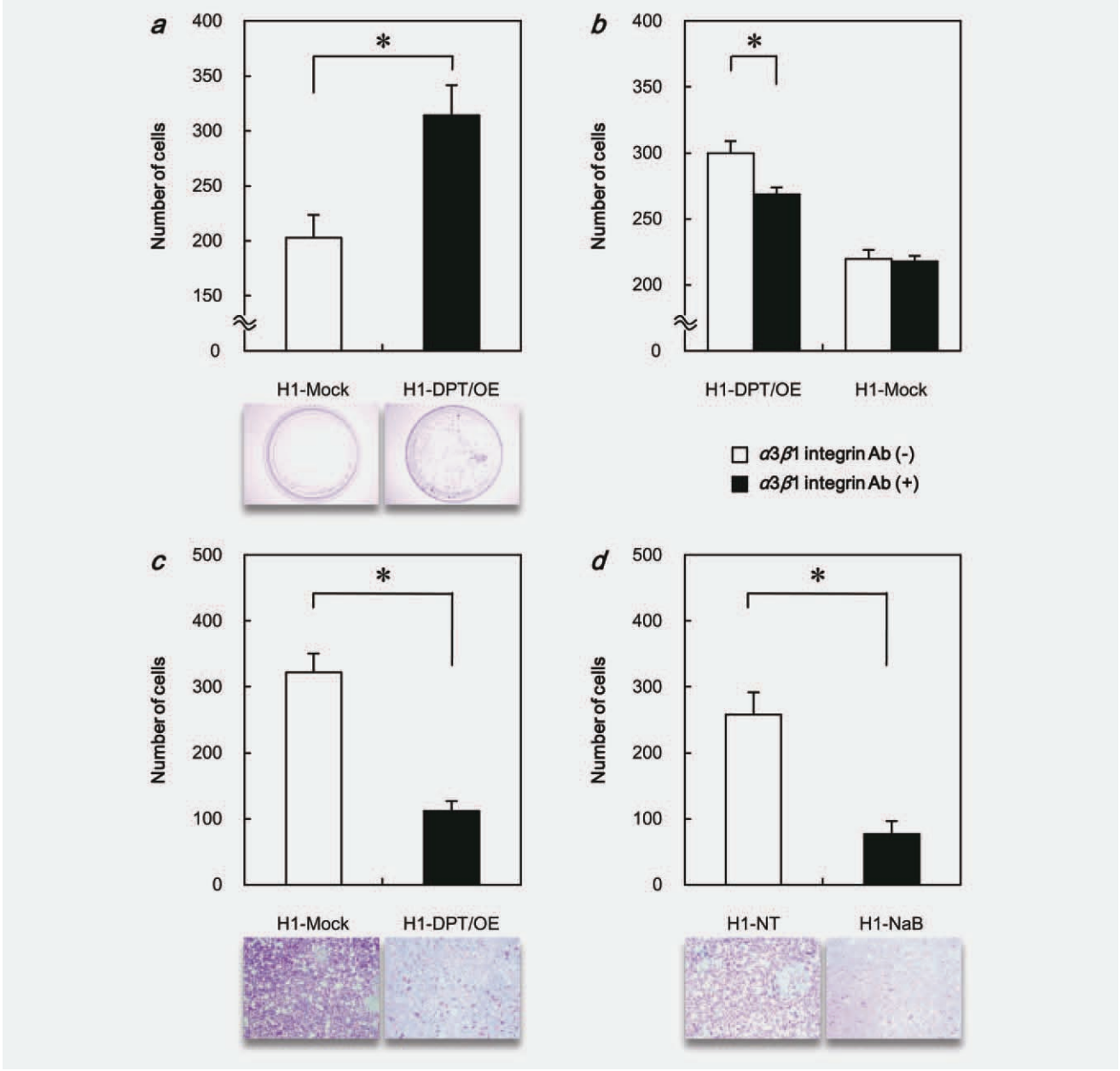

Figure 4. Functional analyses of DPT-overexpressed cells (H1). Experiments were performed twice in triplicate. Data are expressed as the means ± SEM. (*a*) Increased adhesion by DPT-overexpressed cells. The number of DPT-overexpressed cells attached to the dishes was increased compared with mock-transfected cells (*$p < 0.05$, Mann–Whitney's *U* test). (*b*) Inhibition of cellular adhesion by α3β1 integrin monoclonal antibody. The number of DPT-overexpressed cells attached to the dishes was decreased by treatment with α3β1 integrin monoclonal antibody (*$p < 0.05$, Mann–Whitney's *U* test). (*c*) Decreased invasiveness of DPT-overexpressed cells. The number of DPT-overexpressed cells entering through the pores was decreased compared with mock-transfected cells (*$p < 0.05$, Mann–Whitney's *U* test). (*d*) Decreased invasiveness after NaB treatment. The number of NaB-treated cells entering through the pores was decreased in the cells treated for 24 hr compared to the untreated cells (NT). (*$p < 0.05$, Mann–Whitney's *U* test).

staining. The stained cells were determined in at least five random fields at 400× magnification in each section. We counted 300 cells per the one field of vision. The staining intensity (1, Weak; 2, Moderate; 3, Intense) and the number of positive cells in the field of vision were then multiplied for calculation of IHC score. Calculation formula of IHC score is as follows, IHC score = 1 × (the number of Weak stained cells in the field) + 2 × (the number of Moderate stained cells in the field) + 3 × (the number of Intense stained cells in the field). Cases with a score exceeding 115.7 (the lowest score for normal tissue) were defined as DPT-positive. Two independent pathologists were masked to the patients' clinical status.

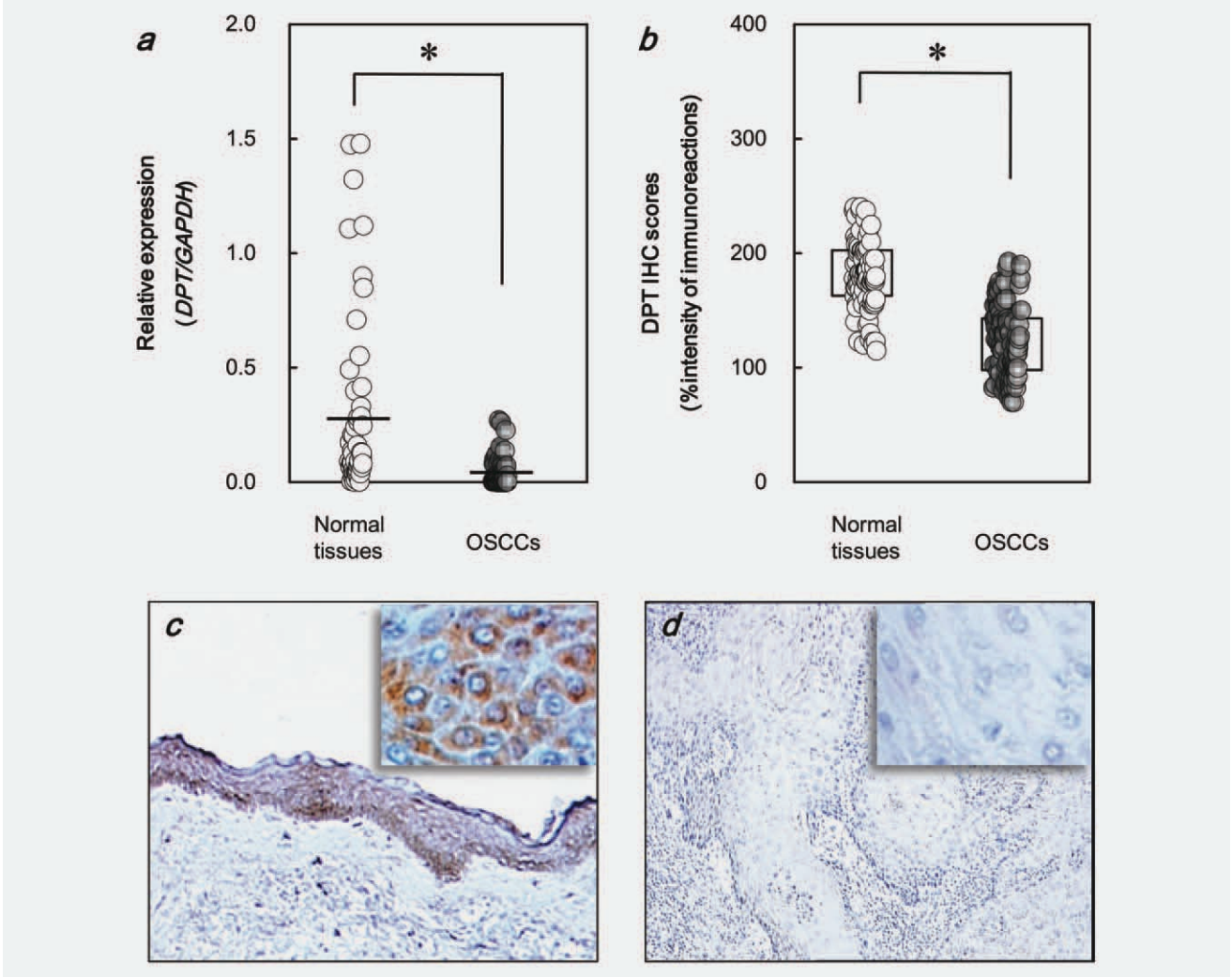

Figure 5. Evaluation of DPT expression in primary OSCCs. (*a*) qRT-PCR analysis showed that *DPT* mRNA expression was downregulated in 68 (70%) of 97 primary OSCCs compared with the matched normal oral tissues. The relative mRNA levels in the normal oral tissues and primary OSCCs ranged from 4.9×10^{-5} to 1.5 (median, 0.1) and 3.5×10^{-4} to 0.343 (median, 0.002), respectively (*$p < 0.05$, Mann–Whitney's *U* test). Data are expressed as the means ± SEM in triplicate. (*b*) The DPT IHC scores of normal oral tissues and OSCCs ranged from 115.7 to 240.5 (median, 178.8) and 70.0 to 192.5 (median, 120.7), respectively. The IHC scores in primary OSCCs were significantly (*$p < 0.05$; Mann–Whitney's *U* test) lower than in normal oral tissues. Representative IHC results for DPT protein in normal oral tissue (*c*) and primary OSCC (*d*) (×100 magnification, inset; ×400). Strong DPT immunoreactivity was detected in normal oral tissues, whereas OSCCs showed almost negative immunostaining.

Statistical analysis

The statistical significance of the DPT expression levels was evaluated using the Fisher's exact test or the Mann–Whitney *U* test. $P < 0.05$ was considered statistically significant. The data are expressed as the mean ± SEM.

Results

Evaluation of DPT mRNA and protein expression in OSCC-derived cell lines

To investigate mRNA and protein expression of DPT identified as a cancer-related gene in our previous microarray data,[21] we performed qRT-PCR and immunoblotting analysis using six OSCC-derived cell lines (HSC-2, HSC-3, HSC-4, H1, Sa3 and Ca9-22). *DPT* mRNA was significantly (Fig. 1*a*, *$p < 0.05$) downregulated in all OSCC-derived cell lines compared to the HNOKs. Representative results of the immunoblotting analysis are shown in Figure 1*b*. The molecular weight of the DPT was 22 kDa. A significant decrease in DPT protein expression was observed in all OSCC-derived cell lines compared to the HNOKs.

Effects of NaB on DPT expression

OSCC-derived cells were treated with NaB to assess the effects of an HDAC inhibitor on DPT expression. In H1 (Fig.

Table 1. Correlation between DPT expression and clinical classification in OSCCs

		Results of immunostaining		
		No. of patients (%)		
Clinical classification	Total	DPT (−)	DPT (+)	*P* value
Age at surgery (years)				
<60	29	13 (45)	16 (55)	0.301
≥60, <70	21	11 (52)	10 (48)	
≥70	47	16 (34)	31 (66)	
Gender				
Male	58	23 (40)	35 (60)	0.700
Female	39	17 (44)	22 (56)	
T-primary tumor				
T1	13	2 (15)	11 (85)	0.192
T2	47	21 (45)	26 (55)	
T3	23	11 (48)	12 (52)	
T4	14	6 (43)	8 (57)	
N-regional lymph node				
N (−)	63	21 (33)	42 (67)	0.031*
N (+)	34	19 (56)	15 (44)	
Stage				
I	11	2 (18)	9 (82)	0.175
II	32	14 (44)	18 (56)	
III	22	8 (36)	14 (64)	
IV	32	16 (50)	16 (50)	
Histopathologic type				
Well	58	25 (43)	33 (57)	0.869
Moderately	33	11 (33)	22 (67)	
Poorly	6	4 (67)	2 (33)	
Tumor site				
Gingiva	28	13 (46)	15 (54)	0.324
Tongue	50	20 (40)	30 (60)	
Buccal mucosa	11	5 (45)	6 (55)	
Oral floor	7	2 (29)	5 (71)	
Oropharynx	1	0 (0)	1 (100)	

*$p < 0.05$.

2*a*) and Sa3 (Fig. 2*b*) cells, *DPT* was upregulated treated with NaB for 12 and 24 hr compared to untreated (*$p < 0.05$).

Establishment of DPT-transfected cells

OSCC-derived cells (H1 and Sa3) transfected with a human DPT stable expression vector were cloned. To assess DPT mRNA and protein expression in DPT-transfected cells, we performed qRT-PCR and immunoblotting analysis. The *DPT* mRNA expression in DPT-transfected cells was significantly (Fig. 3*a*, **$p < 0.001$) higher than in mock-transfected cells. The DPT protein levels in the DPT-transfected cells also increased significantly (Fig. 3*b*, **$p < 0.001$) compared to the mock-transfected cells.

Functional analyses of DPT-overexpressed cells

To investigate the effects of DPT overexpression on cellular proliferation, cellular growth was monitored for 7 days, and no difference was found between the DPT-overexpressed cells and mock-transfected cells (data not shown). We performed adhesion and invasiveness assays to determine the biologic effects in DPT-overexpressed H1 (Fig. 4) and Sa3 (Supporting Information Fig. 1) cells. The number of cells attached to the dishes was counted after crystal violet/methanol staining. Cellular adhesion increased significantly in the DPT-overexpressed cells compared to the mock-transfected cells (Fig. 4*a* and Supporting Information Fig. 1*a*, *$p < 0.05$). DPT plays a role as a cellular adhesion molecule in epidermal cells through α3β1 integrin.[20] We therefore examined whether the adhesion activity of DPT-overexpressed cells could be varied by blocking of α3β1 integrin. Adhesion of DPT-overexpressed H1 and Sa3 cells was inhibited by α3β1 integrin monoclonal antibody (Fig. 4*b* and Supporting Information Fig. 1*b*, *$p < 0.05$). The number of DPT-overexpressed cells that entered through the pores also significantly decreased compared to the mock-transfected cells (Fig. 4*c* and Supporting Information Fig. 1*c*, *$p < 0.05$). Similarly, NaB-treated cells showed decreased invasiveness (Fig. 4*d* and Supporting Information Fig. 1*d*, *$p < 0.05$).

Evaluation of DPT expression in primary OSCCs

We measured the *DPT* mRNA expression levels in primary OSCCs and paired normal oral tissues from 97 patients. Similar to the data from the OSCC-derived cell lines, qRT-PCR analysis showed that *DPT* mRNA expression was downregulated in 68 (70%) of 97 primary OSCCs compared to the matched normal oral tissues. The relative mRNA levels in the normal oral tissues and primary OSCCs ranged from 4.9×10^{-5} to 1.5 (median, 0.1) and 3.5×10^{-4} to 0.343 (median, 0.0016), respectively (Fig. 5*a*, *$p < 0.05$). We analyzed the DPT protein expression in primary OSCCs using the IHC scoring system.[22] The DPT IHC scores of normal oral tissues and OSCCs ranged from 115.7 to 240.5 (median, 178.8) and 70.0 to 192.5 (median, 120.7), respectively. The IHC scores in primary OSCCs were significantly (Fig. 5*b*, *$p < 0.05$) lower than in normal oral tissues. Representative IHC results for DPT protein in normal oral tissue and primary OSCCs are shown in Figures 5*c* and 5*d*. Strong DPT immunoreactivity was detected in the cytoplasm of the basal and prickle cell layers in normal tissue, whereas the OSCCs showed almost negative immunostaining. The correlations between the clinicopathologic characteristics of the patients with OSCC and the status of the DPT protein expression using the IHC scoring system are shown in Table 1. Among the clinical classifications, cells with DPT-negative expression exhibited more

regional lymph node metastasis compared to cells with DPT-positive expression (*$p < 0.05$).

Discussion

The present study showed that DPT, which is an essential component of the ECM, plays an important role in regulating tumor invasiveness and metastasis in OSCCs and that could be not only a novel diagnostic marker of regional lymph node metastasis but also an emerging potential target for therapeutic intervention in the treatment of OSCCs. We report here that OSCCs are neoplasms comprised cells with notable downregulation of DPT.

The ECM protein had been thought to contribute to structural aspects.[7] DPT is one of the noncollagenous ECM proteins. DPT-deficient mice exhibit a phenotype of Ehlers–Danlos syndrome, with poorly organized collagen microfibrils and susceptibility of the skin to mechanical shear stress.[23] Thus, DPT also plays an indispensable role in maintaining functional tissue integrity. Recent advances have highlighted the importance of the cell-ECM interactions. The ECM is a complex and functional networks that can interact simultaneously with cell surface receptors. In the ECM, endostatin, a type of collagen, strongly inhibits angiogenesis and would be a key molecule that controls cancer invasiveness.[24] Decorin, a small leucine-rich proteoglycan and binding partner of DPT, causes significant growth suppression of breast cancer cells and inhibits its metastases to the lungs as a result of decreased tumor size, which leads to reduced tumor invasiveness and metastatic spread.[7,25,26] ECM degrading enzyme, SNC19/ST14, decreases ability of cellular adhesion and increased cellullar invasiveness *in vitro*.[27] These reports indicated that the ECM functions are not limited only to a structural role, but may also affect multiple and diverse functions for cancer cells.

Chromatin/histone modifications are one of the molecular mechanisms that mediate epigenetic regulation.[28] Of them, HDACs, the chromatin-modifying enzymes, remove the acetyl groups, leading to chromatin condensation and repression of transcription.[29] It has also been established that an HDAC inhibitor is involved in the expression of several genes, including ECM and its degrading enzymes.[30,31] In the present study, after treatment of OSCC-derived cells with the HDAC inhibitor, DPT transcription was restored completely. Meanwhile, no significant restoration of DPT expression was observed in a demethylating assay using DNA methyltransferase inhibitor, 5-aza-2′-deoxycytidine (data not shown). These findings indicated that DPT expression in OSCC-derived cells was controlled by epigenetic silencing of chromatin/histone deacetylation.

The cellular adhesion activity of DPT had been reported in fibroblasts, neurogenic cells and epidermal HaCaT cells.[20,32] Consistent with those studies, our data indicated that DPT-overexpressed cells acquired self-sustained cellular adhesion and decreased invasiveness phenotypes. The α3β1 integrin antibody has previously been shown to block adhesion to DPT in HaCaT cells.[20] We therefore examined whether the same response could be observed for DPT-overexpressed cells. Preincubation with the α3β1 integrin monoclonal antibody had inhibitory effect on α3β1 integrin-DPT interactions and resulted in decreased adhesive capacity. Moreover, several studies have shown that DPT may interact with collagen, decorin and TGF-β.[7] Although the biologic meaning of these molecular interactions remains to be determined, the DPT seems to have complex regulatory mechanisms, especially for oral cancer metastasis.

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