Biochemical and Biophysical Research Communications 457 (2015) 71–76

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Decorin in human oral cancer: A promising predictive biomarker of S-1 neoadjuvant chemosensitivity

Atsushi Kasamatsu [a,b,*], Katsuhiro Uzawa [a,b,*], Yasuyuki Minakawa [a], Shunsaku Ishige [a], Hiroki Kasama [a], Yosuke Endo-Sakamoto [b], Katsunori Ogawara [b], Masashi Shiiba [c], Yuichi Takiguchi [c], Hideki Tanzawa [a,b]

[a] Department of Oral Science, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan
[b] Department of Dentistry and Oral–Maxillofacial Surgery, Chiba University Hospital, Chiba 260-8670, Japan
[c] Medical Oncology, Graduate School of Medicine, Chiba University, Chiba 260-8670, Japan

ARTICLE INFO

Article history:
Received 16 December 2014
Available online 27 December 2014

Keywords:
Oral squamous cell carcinoma
Decorin
S-1
Chemoresistant
Neoadjuvant chemotherapy
AKT pathway

ABSTRACT

We reported previously that decorin (DCN) is significantly up-regulated in chemoresistant cancer cell lines. DCN is a small leucine-rich proteoglycan that exists and functions in stromal and epithelial cells. Accumulating evidence suggests that DCN affects the biology of several types of cancer by directly/indirectly targeting the signaling molecules involved in cell growth, survival, metastasis, and angiogenesis, however, the molecular mechanisms of DCN in chemoresistance and its clinical relevance are still unknown. Here we assumed that DCN silencing cells increase chemosusceptibility to S-1, consisted of tegafur, prodrug of 5-fluorouracil. We first established DCN knockdown transfectants derived from oral cancer cells for following experiments including chemosusceptibility assay to S-1. In addition to the *in vitro* data, DCN knockdown zenografting tumors in nude mice demonstrate decreasing cell proliferation and increasing apoptosis with dephosphorylation of AKT after S-1 chemotherapy. We also investigated whether DCN expression predicts the clinical responses of neoadjuvant chemotherapy (NAC) using S-1 (S-1 NAC) for oral cancer patients. Immunohistochemistry data in the preoperative biopsy samples was analyzed to determine the cut-off point for status of DCN expression by receiver operating curve analysis. Interestingly, low DCN expression was observed in five (83%) of six cases with complete responses to S-1 NAC, and in one (10%) case of 10 cases with stable/progressive disease, indicating that S-1 chemosensitivity is dramatically effective in oral cancer patients with low DCN expression compared with high DCN expression. Our findings suggest that DCN is a key regulator for chemoresistant mechanisms, and is a predictive immunomarker of the response to S-1 NAC and patient prognosis.

1. Introduction

S-1 is an oral anticancer drug comprised of tegafur, a prodrug of 5-fluorouracil (5-FU); 5-chloro-2,4-dihydroxypyridine (CDHP); and potassium oxonate (Oxo). CDHP reversibly inhibits 5-FU degradation by dihydropyrimidine dehydrogenase, resulting in prolonged high serum concentrations of 5-FU, thereby enhancing the antitumor activity of tegafur. Oxo is distributed selectively to the gastrointestinal tract, where high concentrations inhibit orotate phosphoribosyltransferase, which phosphorylates 5-FU to an active metabolite in humans. Inhibition of this enzyme reduces gastrointestinal toxicity [1–3].

Neoadjuvant chemotherapy (NAC) improves treatment results by down-staging tumors before treatment, surgery, chemotherapy, and radiotherapy. Several groups have reported that NAC with S-1 (S-1 NAC) was used in patients with advanced gastric cancer [4,5]. However, no convincing evidence has been obtained regarding the contribution of S-1 NAC to improved survival [5]. In our S-1 NAC study of patients with oral squamous cell carcinoma (OSCC) [6], the 61.2% response rate (complete and partial responses [RR]) was higher than reported previously [7,8]. We also found that the RR to S-1 NAC in cases with lymph node metastasis was significantly lower than in cases without metastasis, suggesting that the RR to S-1 NAC may predict regional lymph node metastasis of OSCC [6]. Although S-1 has highly effective antitumor activity and low toxicity in patients with OSCC, some patients do not respond to S-1 NAC and the tumors grow. If we determine the chemosensitivity of S-1 before administration of NAC, we can provide appropriate treatments.

* Corresponding authors at: Department of Oral Science, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan. Fax: +81 43 226 2300.
E-mail addresses: kasamatsua@faculty.chiba-u.jp (A. Kasamatsu), uzawak@faculty.chiba-u.jp (K. Uzawa).

http://dx.doi.org/10.1016/j.bbrc.2014.12.093
0006-291X/

Decorin (DCN) is a prototype member of an expanding family of small leucine-rich proteoglycans [9] that are related structurally and play major roles in organization of the extracellular matrix (ECM) and regulation of cellular behavior [10]. *DCN* may be a tumor suppressor gene that is inactivated in cancer cells during malignant formation in several cancer types [11]. In contrast, low DCN expression in breast cancer is associated with worse prognosis [12]. Therefore, DCN has pivotal roles in various situations. Our previous microarray data showed that DCN is up-regulated in chemoresistant cancer cell lines [13]. However, the molecular status of DCN in chemoresistance and its clinical relevance remain unknown. In the current study, we found that DCN controls the mechanism of chemosensitivity *in vitro* and *in vivo* and that DCN may be a predictive biomarker of the response to S-1 NAC in patients with OSCC.

2. Materials and methods

2.1. Cell lines

We previously established the chemoresistant cell line, Sa3-R, from its parental cancer cell line, Sa3 (human OSCC cell line) [14–16]. The Sa3 cell line was purchased from the RIKEN Bio-Resource, Tsukuba, Japan. The cell cultures were performed as previously described [14–16].

2.2. cDNA preparation

Trizol Reagent (Invitrogen, Carlsbad, CA, USA) was used to isolate total RNA from cells and reverse-transcribed by Ready-to-Go You-Prime first-strand beads (GE Healthcare, Buckinghamshire, UK) and Oligo (dT) primer (Invitrogen).

2.3. Gene expression analysis

Quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) was performed using the LightCycler 480 system (Roche Diagnostics GmbH, Mannheim, Germany) with the following primers to evaluate the DCN expression levels: DCN, forward 5′-GGAG ACTTTAAGAACCCTGAAGAACC-3′ (703–727) and reverse 5′-CGTT CCAACTTCACCAAAGG-3′ (787–806). PCR reactions were performed as previously described [17]. The target gene transcript amounts were estimated from the respective standard curves and normalized to the *glyceraldehyde-3-phosphate dehydrogenase* (GAPDH, forward 5′-CATCTCTGCCCCCTCTGCTGA-3′ and reverse 5′-GGATGAC CTTGCCCACAGCCT-3′) transcript amounts determined in corresponding samples.

2.4. Immunoblot analysis

Protein was extracted as previously described [17]. Protein extracts were first electrophoresed on 4–12% Bis-Tris gel and then transferred to nitrocellulose membranes (Invitrogen) and blocked for 1 h at room temperature in Blocking One (Nacalai Tesque, Kyoto, Japan). 0.1% Tween 20 in TBS was used to wash the membrane three times; the membranes then were incubated overnight at 4 °C with goat anti-DCN polyclonal antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), rabbit anti-AKT polyclonal antibody (Abgent, San Diego, CA, USA), rabbit anti-phosphorylated AKT (p-AKT) polyclonal antibody (Santa Cruz Biotechnology), rabbit anti-proliferating cell nuclear antigen (PCNA) polyclonal antibody (Santa Cruz Biotechnology), or rabbit anti-cleaved caspase-3 polyclonal antibody (Cell Signaling Technology, Danvers, MA, USA). The membranes were washed again and incubated with an anti-goat or anti-rabbit IgG horseradish peroxidase conjugate (Promega, Madison, WI, USA) as secondary antibodies for 1 h at room temperature. Membrane detection was performed as previously described [17].

2.5. shRNA

The cells were transfected stably with DCN shRNA (shDCN) or shRNA control (shMock) plasmids (Santa Cruz Biotechnology) using Lipofectamine LTX and Plus Reagents (Invitrogen). The stable shDCN and shMock cells were isolated using a culture medium containing 2 μg/ml puromycin (Santa Cruz Biotechnology).

2.6. Cellular growth

To determine the effect of DCN knockdown on cellular proliferation, we monitored the cellular growth for 168 h and counted the number of cells every 24 h. The cells were trypsinized and counted using a hemocytometer at the indicated time points.

2.7. Chemosensitivity assay

The cells were seeded at a concentration of 2×10^3 in DMEM containing 10% FBS in all wells of a 96-well plate. Twenty-four hours later, a medium containing various concentrations of 5-FU (6.25, 12.5, 25, 50, 100, 200, 400, 800, and 1600 μM) replaced the culture medium. After a 72-h incubation, a cell viability assay was performed using the Cell Counting kit-8 (Dojindo, Kumamoto, Japan).

2.8. Chemosensitivity assay in nude mice zenografts

The transfectants derived from the Sa3-R cells (2×10^7 cells) were injected subcutaneously into the back of female athymic nude mice (BALB/cAnNcrj-nu/nu) obtained from Charles River Japan Inc. (Yokohama, Kanagawa, Japan). The animals were cared for and treated according to institutional guidelines. S-1 treatment started when the tumors reached 100–150 mm^3 in size. Seven mice in each of five treatment group were given oral S-1 (2 mg/kg on days 0–14.) The treatment groups were as follows: control ($n = 7$), shMock ($n = 7$), shDCN ($n = 7$), shMock with S-1 ($n = 7$), and shDCN with S-1 ($n = 7$). The tumoral volume and the body weight of the mice were recorded twice weekly over the course of 42 days. The longest perpendicular tumor diameters were measured using calipers on alternate day to estimate the tumoral volume using the following formula: $4\pi/3 \times (\text{width}/2)2 \times (\text{length}/2)$.

2.9. Immunohistochemistry

Immunohistochemistry (IHC) using the primary antibody was performed as previously described [17–19]. To quantify the DCN protein expression in those components, we used the previously described IHC score system [17–19].

2.10. TUNEL assay

Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed using an *in situ* apoptosis detection kit (TaKaRa, Tokyo, Japan). The number of TUNEL-positive cells was detected and counted by light microscopy.

2.11. Patients and treatment schedules

Sixteen patients who had previously been diagnosed with OSCC at the Department of Oral–Maxillofacial Surgery, Chiba University Hospital, were enrolled in the study. The Institutional Review Board of Chiba University approved the study protocol (No. 236).

All patients provided written informed consent before entry into the study. To suppress tumoral growth preoperatively, the patients were treated with S-1 NAC before the primary tumor was resected with simultaneous neck dissection as previously described [6].

2.12. Evaluation of treatment effects

The tumoral size and histopathologic findings from the biopsy specimens after treatment with S-1 NAC were measured to determine the extent of the disease. Each neoplastic tissue specimen was histopathologically diagnosed according to the World Health Organization criteria by the Department of Pathology, Chiba University Hospital. The Response Evaluation Criteria in Solid Tumors guidelines were used to evaluate the response to treatment.

2.13. Statistical analysis

The data are expressed as the mean ± standard error of the mean (SEM) from three assays. Receiver operating characteristic (ROC) curve analysis determined the optimal cut-off points. Statistical analyses were performed using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA).

3. Results

3.1. Chemosensitivity in DCN knockdown cells

We previously reported overexpression of DCN in cis-diamminedichloroplatinum- and 5-FU-resistant OSCC cell line (Sa3-R) [13]. Since then, we examined whether DCN regulates chemosensitivity in DCN knockdown cells. qRT-PCR data showed that the *DCN* mRNA expression in DCN knockdown (shDCN) cells was significantly ($P < 0.05$) lower than in control (shMock) cells (Fig. 1A). DCN protein expression also decreased in shDCN cells compared with shMock cells (Fig. 1B). To evaluate the effect of DCN knockdown on cellular growth, we performed a cellular proliferation assay. The growth curves of both transfectants were similar, indicating that down-regulation of DCN did not affect cellular growth (Fig. 1C). To determine whether DCN knockdown affects the tumoral response to chemotherapy, we carried out a chemosensitivity assay of the transfectants using 5-FU, a component of S-1. The half maximal inhibitory concentration (IC_{50}) data indicated that the 5-FU-resistance level of the shDCN cells (IC_{50}, 16.4 μM) was 8.7-fold lower than that of the shMock cells (IC_{50}, 142.2 μM) (Fig. 1D). These data indicated that silencing of DCN increases 5-FU chemosensitivity.

3.2. Immunoblot analysis of DCN knockdown cells

To investigate the effect of 5-FU on cellular proliferation, apoptosis, and the AKT pathway in shMock cell and shDCN cells, we performed immunoblot analysis using PCNA, cleaved caspase-3, AKT, and p-AKT antibodies (Fig. 1E). PCNA and p-AKT are down-regulated and cleaved caspase-3 is up-regulated in shDCN cells after treatment with 5-FU. The data showed that low proliferation, high apoptosis, and dephosphorylation of AKT were significant ($P < 0.05$) in shDCN cells after treatment with 5-FU (Fig. 1F).

3.3. Antitumoral activity of S-1 in DCN knockdown zenografts

The transfectants (shDCN and shMock) and their parental cells (Sa3-R) were inoculated subcutaneously into female athymic nude mice and allowed to grow to a mean volume of 100 mm^3. The mice with shDCN or shMock zenografts were treated with S-1. Consistent with the *in vitro* cellular proliferation data, we found similar zenograft growth between shDCN and shMock cells (Fig. 2A). The response of the shDCN zenografts to S-1 was enhanced significantly ($P < 0.01$) compared with the S-1-treated shMock zenografts (Fig. 2A). S-1 reduced the mean volume of the shDCN zenografts by 48.0% (1216.2 ± 222.9 mm^3 compared with 2338.5 ± 114.4 mm^3 in

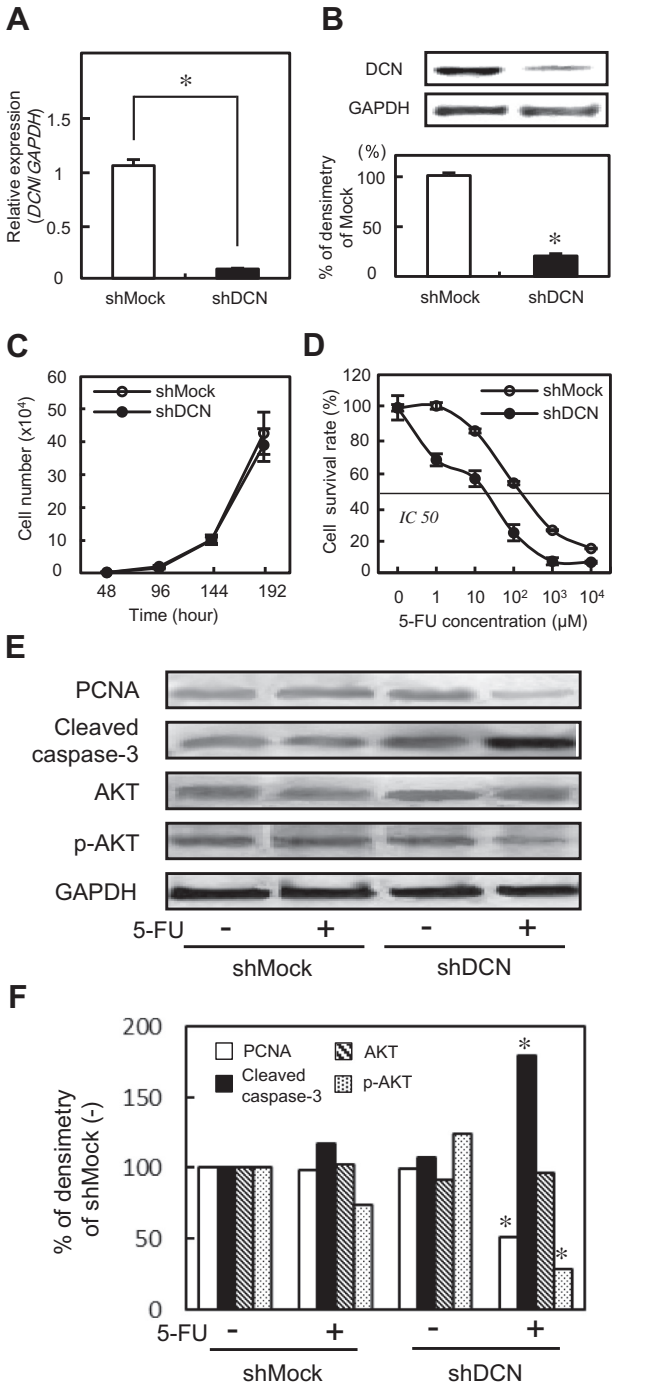


Fig. 1. Chemosensitivity in DCN knockdown cells. (A) Significant down-regulation of *DCN* mRNA is observed in shDCN cells compared with that in shMock cells (*$P < 0.05$). (B) The DCN protein is significantly down-regulated in shDCN cells. Densitometric DCN protein data are normalized to GAPDH protein expression (*$P < 0.05$). (C) Similar cellular growth curves are seen in shDCN and shMock cells. (D) The IC_{50} values of 5-FU for the shDCN (●) and shMock (○) cells are 16.4 μM and 142.2 μM, respectively. (E) Representative immunoblot analysis data of PCNA, cleaved caspase-3, AKT, and p-AKT proteins in shDCN and shMock cells. (F) PCNA and p-AKT are down-regulated and cleaved caspase-3 is up-regulated in shDCN cells after treatment with 5-FU (*$P < 0.05$).

the control groups) (Fig. 2A). We found that the average murine body weights in the S-1 treatment groups never decreased below that of the control groups (untreated parental cells, shMock cells, and shDCN cells) at any time during the experiments (Fig. 2B).

3.4. Immunohistochemical analysis of DCN knockdown zenografts

Cellular proliferation and apoptosis can regulate tumoral size at any given time. Therefore, we performed IHC of the zenografting tumoral tissues to measure PCNA expression and the TUNEL assay to detect apoptosis. Decreased proliferation (PCNA) (Fig. 3A) and enhanced apoptosis (TUNEL) (Fig. 3B) were most pronounced in the shDCN zenografts treated with S-1 (Fig. 3C) ($P < 0.05$), which suggested that the enhanced S-1 chemotherapeutic effects in the shDCN zenografts resulted from reduced tumor cell proliferation and increased apoptosis.

Since the AKT signaling pathway plays a critical role in cellular survival [20], we performed IHC using AKT and phosphorylated AKT antibodies for the zenografts (Fig. 3D and E). The AKT expression was similar in the shDCN and shMock zenografts with and without S-1 treatment (Fig. 3D and F). Interestingly, phosphorylated AKT expression in the shDCN zenografts treated with S-1 was significantly ($P < 0.05$) lower than in the shDCN zenografts without S-1 treatment and shMock zenografts with and without S-1 treatment (Fig. 3E and F).

3.5. Patient characteristics and responses to S-1 NAC

The primary site of the tumor was the tongue in the 13 men and three women (age range, 53-83 years; mean, 65.8 years). Six (37.5%) had complete responses (CRs) and 10 (62.5%) cases had stable or progressive disease (SD/PD).

3.6. Evaluation of DCN expression in biopsy samples of OSCCs

We analyzed the DCN protein expression in biopsy samples before treatment with S-1 NAC for OSCCs using the IHC scoring system. Representative IHC results for DCN protein in cases with a CR and those with SD/PD after S-1 NAC are shown in Fig. 4A and B, respectively. Strong DCN immunoreactivity was detected in the cytoplasm and membranes in cases with SD/PD treated with S-1 NAC, and cases with a CR treated with S-1 NAC showed weak immunostaining. The DCN IHC scores in cases with a CR and SD/PD ranged from 9 to 145 (median, 67.5) and 48 to 199 (median, 155.5), respectively. The IHC scores in the cases with a CR were significantly ($P < 0.05$) lower than in cases with SD/PD (Fig. 4C).

The optimal threshold value was assessed using ROC curve analysis (Fig. 4D). When a cut-off value of 119 (Fig. 4D, open circle) was used for the DCN expression level, the area under the ROC curve was 0.889 (95% confidence interval, 0.754–1.000, $P < 0.001$), suggesting that DCN expression is a biomarker for evaluating the chemosensitivity of S-1 NAC. Low DCN expression was seen in five (83%) of six cases with a CR treated with S-1 NAC and in one (10%) of 10 cases with SD/PD treated with S-1 NAC (Fig. 4E).

4. Discussion

Because we previously found that *DCN* is one of the up-regulated genes in the chemoresistant OSCC cell lines [13], we hypothesized that DCN may function in a chemoresistant role in OSCC. The current study showed that silencing DCN significantly increased the chemosusceptibility to S-1 in OSCC cells and their zenografting tumors via decreased AKT phosphorylation. We next investigated whether DCN expression predicts the response to S-1 NAC in patients with OSCC. ROC curve analysis showed that S-1 chemosensitivity in patients with OSCC with low DCN expression was significantly greater than in those with high DCN expression (Fig. 4E). The data supported the idea that measuring DCN expression may predict the responses to S-1 NAC.

DCN is the most well-known member of the family of small proteoglycans characterized by core proteins with leucine-rich repeats. DCN is expressed in many different cellular types and has been implicated in the control of ECM formation [9]; decreased DCN expression especially results in fragile connective tissue structures [21]. Abundant DCN expression may lead to an over-organized ECM that can trap chemotherapeutic agents to attenuate anticancer activity [10,22]. Therefore, DCN-mediated ECM alterations may alter the sensitivity of cancer cells to chemotherapy.

DCN is also a novel biologic ligand of epidermal growth factor receptor (EGFR) [10,23] in that it binds to the EGFR and increases its phosphorylation, leading to activation of the AKT pathway [24]. DCN protects cells from apoptosis through the AKT pathway [25], which plays a critical regulatory role in the cellular survival/death process [20]. AKT plays a pivotal role in signaling pathways downstream of phosphatidylinositol 3-kinase, regulating fundamental processes such as cellular survival, proliferation, dif-

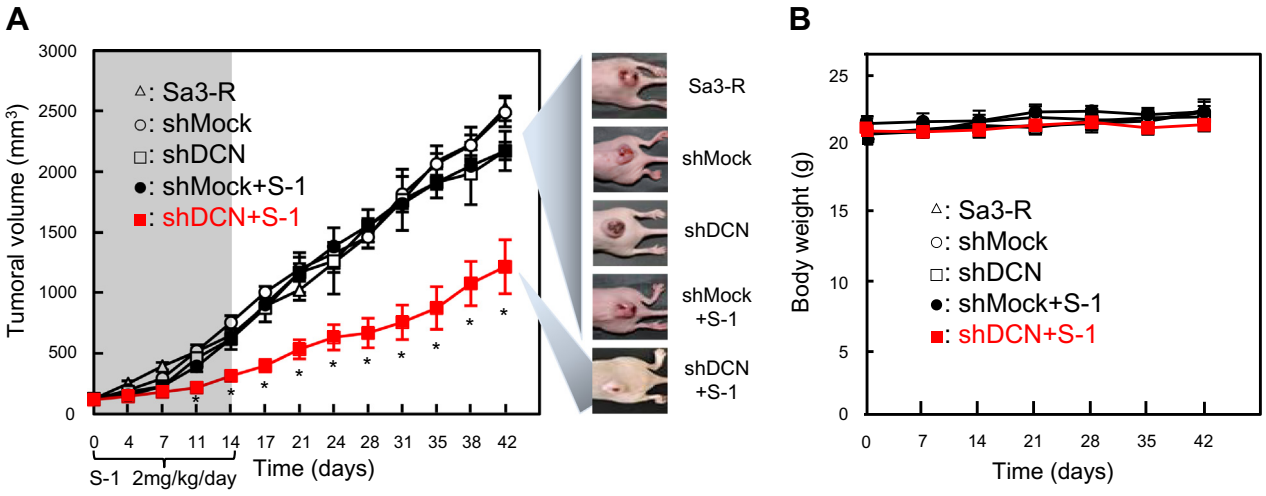


Fig. 2. Antitumoral activity of S-1 in DCN knockdown zenografts. (A) The response of the shDCN cell zenografts to S-1 treatment is enhanced significantly (*$P < 0.01$) compared to shMock zenografts with S-1 treatment. (B) The average body weights of the mice in the S-1 treatment groups (shMock + S-1 and shDCN + S-1) never decreased below that of the control groups (untreated Sa3-R cells, shMock cells, and shDCN cells) at any time point.

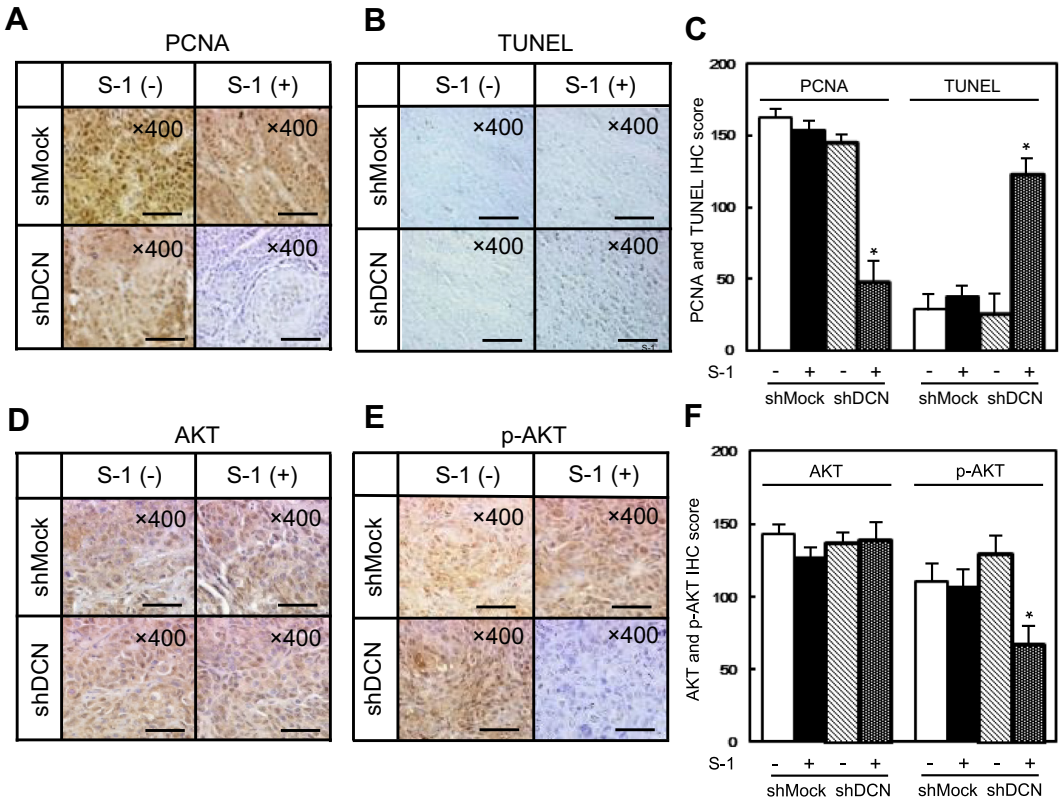
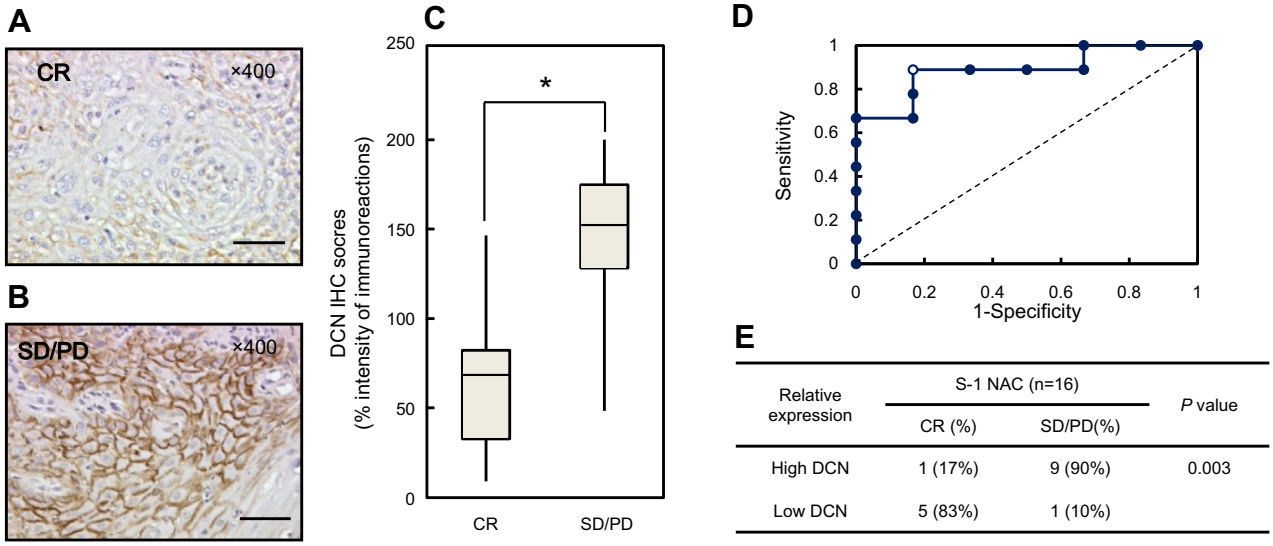


Fig. 3. Immunohistochemical analysis of DCN knockdown zenografts. Decreased proliferation (PCNA, A) and enhanced apoptosis (TUNEL, B) are most pronounced in the shDCN zenografts treated with S-1 (C, $^{*}P < 0.05$). AKT expression is similar in the shDCN and shMock zenografts with or without S-1 treatment (D and F). p-AKT expression in the shDCN zenografts with S-1 treatment is significantly lower than that in the shDCN zenografts without S-1 treatment and shMock zenografts with or without S-1 treatment (E and F) ($^{*}P < 0.05$).



E

Relative expression	S-1 NAC (n=16)		*P* value
	CR (%)	SD/PD(%)	
High DCN	1 (17%)	9 (90%)	0.003
Low DCN	5 (83%)	1 (10%)	

Fig. 4. Evaluation of DCN protein expression in biopsy samples of primary OSCCs. (A and B) Representative IHC results of DCN protein. (A) A case with a CR to S-1 NAC has weak DCN protein expression in the biopsy sample. Scale bars, 50 μm. (B) A case of SD/PD after S-1 NAC has a positive immunoreaction for DCN in the cytoplasm and membranes. Scale bars, 50 μm. (C) The DCN protein expression in CR cases is decreased compared with SD/PD cases ($^{*}P < 0.05$). (D) ROC curves using the IHC scoring system for discriminating the cut-off value between the study groups (sensitivity, 88.9%; specificity, 83.3%; AUC, 0.889). The proposed threshold cut-off value is 119 (open circle). The dotted line indicates the diagonal representing a hypothetical test with no diagnostic discrimination (AUC, 0.5). (E) Correlation between response of S-1 NAC and DCN expression. Low DCN expression is seen in five (83%) of six cases with a CR of S-1 NAC and in one (10%) of 10 cases with SD/PD treated with S-1 NAC.

ferentiation, and metabolism. Increased AKT activation has been associated with increased cellular proliferation in cancer [26] and a poor prognosis in acute leukemia [27]. We showed that DCN knockdown inhibited activation of the AKT pathway by down-regulating phosphorylated AKT after S-1 treatment, leading to increased chemosusceptibility. Consistent with our previous study that cellular proliferation and apoptosis can regulate tumoral size at any given time [28], the current data showed low cellular proliferation and high apoptosis in DCN knockdown zenografts after treatment with S-1 using IHC for PCNA and the TUNEL assay.

The ROC curve is a widely accepted method for selecting an optimal cut-off point for a test and comparing the accuracy of diagnostic tests [29] and is also important because the area under the curve (AUC) reflects the degree to which a test can distinguish between patients with and without disease [30]. Generally, AUC values exceeding 0.9 reflect high accuracy; 0.7–0.9 moderate accuracy; 0.5–0.7 low accuracy; and 0.5 a chance result [30]. The AUC value in the current study was 0.889 (Fig. 4D). These results suggested that the status of DCN protein expression using the IHC scoring system in tumoral tissues might be a potential prognostic predictor of the therapeutic effects of S-1 NAC for treating patients with OSCC, despite the fact that the current study included a small number of clinical samples. We believe that by using more samples, the diagnostic model and increased accuracy of the chemosensitivity predictions can be refined.

In summary, the current report described the expression pattern of DCN in OSCCs, and evaluation of DCN expression by IHC may be another effective tool for identifying patients with OSCC at increased or decreased risk of chemoresistance. DCN also may be a potential novel immunomarker predictive of the prognosis of chemotherapy for patients with OSCC. Furthermore, our data suggested that DCN may be an encouraging molecular target for development of novel combination therapeutic strategies aimed at overcoming chemotherapy resistance in OSCC.

Conflict of interest statement

The authors have no competing interests to declare.

Acknowledgments

We thank Lynda C. Charters for editing this manuscript; Dr. Takao Baba, Chiba University, for assistance with the statistical analysis; and Dr. Fumihiko Uchida, University of Tsukuba, for assistance with the IHC experiments.

References

[1] P. Schoffski, The modulated oral fluoropyrimidine prodrug S-1, and its use in gastrointestinal cancer and other solid tumors, Anticancer Drugs 15 (2004) 85–106.
[2] T. Shirasaka, K. Nakano, T. Takechi, H. Satake, J. Uchida, A. Fujioka, H. Saito, H. Okabe, K. Oyama, S. Takeda, N. Unemi, M. Fukushima, Antitumor activity of 1 M tegafur-0.4 M 5-chloro-2,4-dihydroxypyridine-1 M potassium oxonate (S-1) against human colon carcinoma orthotopically implanted into nude rats, Cancer Res. 56 (1996) 2602–2606.
[3] T. Shirasaka, Y. Shimamato, H. Ohshimo, M. Yamaguchi, T. Kato, K. Yonekura, M. Fukushima, Development of a novel form of an oral 5-fluorouracil derivative (S-1) directed to the potentiation of the tumor selective cytotoxicity of 5-fluorouracil by two biochemical modulators, Anticancer Drugs 7 (1996) 548–557.
[4] M. Kochi, M. Fujii, T. Kaiga, T. Takahashi, Y. Morishita, M. Kobayashi, Y. Kasakura, T. Takayama, FLEP chemotherapy for alpha-fetoprotein-producing gastric cancer, Oncology 66 (2004) 445–449.
[5] M. Yano, H. Shiozaki, M. Inoue, S. Tamura, Y. Doki, T. Yasuda, Y. Fujiwara, T. Tsujinaka, M. Monden, Neoadjuvant chemotherapy followed by salvage surgery: effect on survival of patients with primary noncurative gastric cancer, World J. Surg. 26 (2002) 1155–1159.
[6] H. Yokoe, A. Kasamatsu, K. Ogawara, T. Ishigami, Y. Sato, M. Shiiba, T. Hideki, K. Uzawa, Neoadjuvant chemotherapy with S-1 for patients with oral squamous cell carcinoma, J. Cancer Sci. Ther. 2 (2010).
[7] Y. Inuyama, A. Kida, M. Tsukuda, N. Kohno, B. Satake, Late phase II study of S-1 in patients with advanced head and neck cancer, Gan To Kagaku Ryoho 28 (2001) 1381–1390.
[8] Y. Kimura, N. Kikkawa, S. Iijima, T. Kato, Y. Naoi, T. Hayashi, T. Tanigawa, H. Yamamoto, E. Kurokawa, A new regimen for S-1 therapy aiming at adverse reaction mitigation and prolonged medication by introducing a 1-week drug-free interval after each 2-week dosing session: efficacy and feasibility in clinical practice, Gastric Cancer 6 (Suppl. 1) (2003) 34–39.
[9] R.V. Iozzo, Matrix proteoglycans: from molecular design to cellular function, Annu. Rev. Biochem. 67 (1998) 609–652.
[10] R.V. Iozzo, The biology of the small leucine-rich proteoglycans. Functional network of interactive proteins, J. Biol. Chem. 274 (1999) 18843–18846.
[11] M.A. Nash, M.T. Deavers, R.S. Freedman, The expression of decorin in human ovarian tumors, Clin. Cancer Res. 8 (2002) 1754–1760.
[12] S. Troup, C. Njue, E.V. Kliewer, M. Parisien, C. Roskelley, S. Chakravarti, P.J. Roughley, L.C. Murphy, P.H. Watson, Reduced expression of the small leucine-rich proteoglycans, lumican, and decorin is associated with poor outcome in node-negative invasive breast cancer, Clin. Cancer Res. 9 (2003) 207–214.
[13] Y. Yamano, K. Uzawa, K. Saito, D. Nakashima, A. Kasamatsu, H. Koike, Y. Kouzu, K. Shinozuka, K. Nakatani, K. Negoro, S. Fujita, H. Tanzawa, Identification of cisplatin-resistance related genes in head and neck squamous cell carcinoma, Int. J. Cancer 126 (2010) 437–449.
[14] M. Nakamura, K. Nakatani, K. Uzawa, K. Ono, H. Uesugi, K. Ogawara, M. Shiiba, H. Bukawa, H. Yokoe, T. Wada, S. Fujita, H. Tanzawa, Establishment and characterization of a cisplatin-resistant oral squamous cell carcinoma cell line, H-1R, Oncol. Rep. 14 (2005) 1281–1286.
[15] K. Nakatani, M. Nakamura, K. Uzawa, T. Wada, N. Seki, H. Tanzawa, S. Fujita, Establishment and gene analysis of a cisplatin-resistant cell line, Sa-3R, derived from oral squamous cell carcinoma, Oncol. Rep. 13 (2005) 709–714.
[16] K. Negoro, Y. Yamano, K. Fushimi, K. Saito, K. Nakatani, M. Shiiba, H. Yokoe, H. Bukawa, K. Uzawa, T. Wada, H. Tanzawa, S. Fujita, Establishment and characterization of a cisplatin-resistant cell line, KB-R, derived from oral carcinoma cell line, KB, Int. J. Oncol. 30 (2007) 1325–1332.
[17] Y. Kouzu, K. Uzawa, H. Koike, K. Saito, D. Nakashima, M. Higo, Y. Endo, A. Kasamatsu, M. Shiiba, H. Bukawa, H. Yokoe, H. Tanzawa, Overexpression of stathmin in oral squamous-cell carcinoma: correlation with tumour progression and poor prognosis, Br. J. Cancer 94 (2006) 717–723.
[18] D.P. Lombardi, J. Geradts, J.F. Foley, C. Chiao, P.W. Lamb, J.C. Barrett, Loss of KAI1 expression in the progression of colorectal cancer, Cancer Res. 59 (1999) 5724–5731.
[19] T. Onda, K. Uzawa, Y. Endo, H. Bukawa, H. Yokoe, T. Shibahara, H. Tanzawa, Ubiquitous mitochondrial creatine kinase downregulated in oral squamous cell carcinoma, Br. J. Cancer 94 (2006) 698–709.
[20] L.C. Cantley, The phosphoinositide 3-kinase pathway, Science 296 (2002) 1655–1657.
[21] K.G. Danielson, H. Baribault, D.F. Holmes, H. Graham, K.E. Kadler, R.V. Iozzo, Targeted disruption of decorin leads to abnormal collagen fibril morphology and skin fragility, J. Cell Biol. 136 (1997) 729–743.
[22] Y. Hu, H. Sun, R.T. Owens, J. Wu, Y.Q. Chen, I.M. Berquin, D. Perry, J.T. O'Flaherty, I.J. Edwards, Decorin suppresses prostate tumor growth through inhibition of epidermal growth factor and androgen receptor pathways, Neoplasia 11 (2009) 1042–1053.
[23] D.K. Moscatello, M. Santra, D.M. Mann, D.J. McQuillan, A.J. Wong, R.V. Iozzo, Decorin suppresses tumor cell growth by activating the epidermal growth factor receptor, J Clin Invest 101 (1998) 406–412.
[24] E. Schonherr, C. Sunderkotter, R.V. Iozzo, L. Schaefer, Decorin, a novel player in the insulin-like growth factor system, J. Biol. Chem. 280 (2005) 15767–15772.
[25] J.E. Skeen, P.T. Bhaskar, C.C. Chen, W.S. Chen, X.D. Peng, V. Nogueira, A. Hahn-Windgassen, H. Kiyokawa, N. Hay, Akt deficiency impairs normal cell proliferation and suppresses oncogenesis in a p53-independent and mTORC1-dependent manner, Cancer Cell 10 (2006) 269–280.
[26] Y.H. Min, J.I. Eom, J.W. Cheong, H.O. Maeng, J.Y. Kim, H.K. Jeung, S.T. Lee, M.H. Lee, J.S. Hahn, Y.W. Ko, Constitutive phosphorylation of Akt/PKB protein in acute myeloid leukemia: its significance as a prognostic variable, Leukemia 17 (2003) 995–997.
[27] K. Uzawa, T. Ishigami, K. Fushimi, T. Kawata, K. Shinozuka, A. Kasamatsu, Y. Sakamoto, K. Ogawara, M. Shiiba, H. Bukawa, H. Ito, H. Tanzawa, Targeting fibroblast growth factor receptor 3 enhances radiosensitivity in human squamous cancer cells, Oncogene 30 (2011) 4447–4452.
[28] A.K. Akobeng, Understanding diagnostic tests 3: receiver operating characteristic curves, Acta Paediatr. 96 (2007) 644–647.
[29] J.E. Fischer, L.M. Bachmann, R. Jaeschke, A readers' guide to the interpretation of diagnostic test properties: clinical example of sepsis, Intensive Care Med. 29 (2003) 1043–1051.
[30] J.A. Swets, Measuring the accuracy of diagnostic systems, Science 240 (1988) 1285–1293.