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ORIGINAL ARTICLE

Capecitabine improves cancer cachexia and normalizes IL-6 and PTHrP levels in mouse cancer cachexia models

Kaori Fujimoto-Ouchi · Etsuro Onuma · Masatoshi Shirane · Kazushige Mori · Yutaka Tanaka

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Abstract
Purpose To clarify the potential of parathyroid hormone-related protein (PTHrP) and interleukin-6 (IL-6) as cachectic factors in a colon 26 model and the effects of capecitabine on cancer cachexia as determined by plasma levels of IL-6 and PTHrP and body weight loss.
Methods From two colon 26 sublines-cancer cachectic clone20 and non-cachectic clone5 plasma levels of PTHrP protein and mRNA expression levels in tumor tissues were compared. An IL-6 neutralizing antibody, a PTHrP neutralizing antibody, and capecitabine were administered into mice bearing clone20 and their anticachectic effects evaluated.
Results The plasma level of PTHrP protein in mice bearing clone20 was higher than that in mice bearing clone5. The expression level of PTHrP mRNA was 49-fold higher in tumor tissues of clone20 than of clone5, according to GeneChip® analysis. PTHrP antibody as well as IL-6 antibody suppressed wasting of the body and gastrocnemius and adipose tissue weights. PTHrP antibody suppressed the induction of hypercalcemia but not hypoglycemia or elevation of IL-6, whereas IL-6 antibody suppressed the induction of hypoglycemia but not hypercalcemia or elevation of PTHrP. Capecitabine, a fluorinated pyrimidine anticancer agent, improved body wasting of mice bearing clone20 at a low dose with no reduction of tumor volume. Furthermore, capecitabine lowered the levels of PTHrP and IL-6 in plasma and suppressed hypoglycemia and hypercalcemia in this model. Capecitabine also showed anticachectic effects on cachexia in a cancer model induced by human cervical cancer cell line Y (also known as Yumoto).
Conclusions PTHrP and IL-6 were found to be factors in the development of cachexia in a colon 26 cancer model, and capecitabine improved cancer cachexia by suppressing the plasma levels of IL-6 and PTHrP in colon 26 and Y cachectic models.

Keywords Parathyroid hormone-related protein · Interleukin-6 · Cancer cachexia · Capecitabine · Preclinical model

K. Fujimoto-Ouchi (✉) · M. Shirane · K. Mori · Y. Tanaka
Product Research Department, Kamakura Research Laboratories, Chugai Pharmaceuticals Co., Ltd.,
200 Kajiwara, Kamakura, Kanagawa, 247-8530, Japan
e-mail: ohuchikor@chugai-pharm.co.jp

E. Onuma
Pharma Research Department 3, Kamakura Research Laboratories, Chugai Pharmaceuticals Co., Ltd.,
200 Kajiwara, Kamakura, Kanagawa, Japan

Introduction

Cancer cachexia, which includes disorders of homeostasis such as progressive wasting, weakness, anorexia, and anemia, is commonly seen in cancer patients [3, 21]. Cancer cachexia is associated not only with deterioration of the quality of life but also with shorter survival [3] and poor response to chemotherapy [4]. Elucidation of the mechanism of cachexia induction will help in the search for potential therapeutic interventions for advanced cancer.

Previously, we reported that murine colon 26 induced cancer cachexia [33], and showed that interleukin-6 (IL-6) is essential for cancer cachexia of colon 26, in which treatment with IL-6 antibody prevented the induction of cancer cachexia [8]. However, because the blood level of IL-6 was comparable in both cachectic

and non-cachectic colon 26 sublines [8], we concluded that IL-6 is not the only factor responsible for cachexia. The IL-6 antibody did not suppress the elevation of circulating calcium in this model, and so we hypothesized the presence of an additional factor causing hypercalcemia and cachexia. Parathyroid hormone-related protein (PTHrP) has been reported to cause hypercalcemia [29] and cachexia [14, 25]. Therefore, in the present study, we examined the involvement of PTHrP in a colon 26 cachexia model.

We have also reported that 5′-deoxy-5-fluorouridine (5′-dFUrd), an intermediate of capecitabine, was able to improve cachexia in murine colon 26 carcinoma even at doses that do not reduce the tumor burden [5, 34]. Here we demonstrated the anticachectic effects of capecitabine, a new fluorinated pyrimidine anticancer agent, on cachexia and the reduction of plasma levels of IL-6 and PTHrP in cancer cachectic models.

Materials and methods

Antibodies and chemotherapeutic agents

Rat anti-mouse IL-6 antibody was purified from the ascites fluid of CAnN.Cg-Foxn1<nu>/CrlCrlj nu/nu (BALB-nu/nu) mice bearing hybridoma 6B4-B521 with a Prosep-A protein-A column (Millipore Japan, Tokyo, Japan) after precipitation with 50% saturated ammonium sulfate. Anti-human PTHrP antibody was synthesized in Chugai Pharmaceuticals Co., Ltd. [24]. Human IgG was purchased from MP Biomedicals (Eschwege, Germany) as the negative control for antibodies. All antibodies were diluted with saline. Capecitabine, 5′-dFUrd, and ibandronate were provided by F. Hoffman-La Roche as purified powders. Capecitabine and 5′-dFUrd were dissolved in 40 mM citrate buffer (pH 6.0) containing 5% (w/v) gum Arabic. Ibandronate was dissolved in saline.

Animals

Male 4-week-old CD2F1 (BALB/c × DBA/2 F1) mice were obtained from SLC (Hamamatsu, Japan). Female 5-week-old BALB-nu/nu mice were obtained from Charles River Japan (Yokohama, Japan). All animals were allowed to acclimatize and recover from shipping-related stress for 1 week prior to the study. The health of the mice was monitored daily. Chlorinated water and irradiated food were provided ad libitum, and the animals were kept in a controlled light–dark cycle (12 h–12 h). All animal experiments were performed in accordance with the Guidelines for the Accommodation and Care of Laboratory Animals in Chugai Pharmaceutical Research Center.

Cell lines and culture conditions

Hybridoma producing mIL-6 antibody 6B4 established in the laboratory of Dr. J. van Snick (Ludwig Institute for Cancer Research, Brussels, Belgium) was re-cloned (6B4-B521) and kindly provided by Prof. K. Kumagai (Tohoku University Dental School, Sendai, Japan). 6B4-B521 was maintained in RPMI-1640 supplemented with 10% FBS and 10 ng/ml of murine IL-6. Colon 26 sublines, clone20 and clone5, were established in our laboratory [8]. The parent colon 26 in vivo line was kindly provided by Dr. T. Kataoka (Cancer Chemotherapy Center, Cancer Research Foundation, Tokyo, Japan). The colon 26 sublines were maintained in RPMI-1640 supplemented with 10% (v/v) fetal bovine serum (FBS). Human cervical cancer cell line Y was provided by Dr. Tokita [35] and maintained in BALB-nu/nu mice by subcutaneous (sc) inoculation of tumor pieces. The Y cell line was renamed from Yumoto in response to Dr. Tokita's recommendation.

Cancer cachexia models

Each colon 26 subline was inoculated sc at 1×10^6 cells/mouse into the right flank of the CD2F1 mice. An 8-mm^3 piece of Y was inoculated sc into the right flank of the BALB-nu/nu mice. Tumor volume (V) was estimated from the equation $V = ab^2/2$, where a and b are tumor length and width, respectively. Carcass weight was calculated as the difference in weight between the whole body and the tumor. Tumor weight was estimated by multiplying the tumor volume by a correction factor determined by comparing actual tumor weights with tumor volumes from separate experiments [33].

Treatment of animals

Several days after tumor inoculation, mice were randomly allocated to control and treatment groups and were treated with the test agents (6–10 mice/group). Administration of antibodies was started before wasting in order to examine the suppression of wasting. Antibodies were administered intraperitoneally (ip), twice a week for 1 week. The doses of antibodies and 5′-dFUrd were determined with reference to previous experiments [8, 24, 34]. Administration of capecitabine was started when the carcass weights indicated wasting in order to examine cachexia improvement. Capecitabine was administered orally (po), once a day for 8 or

10 consecutive days. Capecitabine was examined at a molecular dose (0.5 mmol/kg: 180 mg/kg) or at a toxic dose (2/3 MTD: 359 mg/kg) equal to 123 mmol/kg of 5′-dFUrd. Ibandronate was administered sc, once a day for 7 days.

Measurement of parameters

Plasma, gastrocnemius and adipose tissue samples were collected from the treated mice on the next day after the last treatment. Samples were taken between 9 and 12 am to minimize any fluctuation in hormone levels due to the circadian rhythm. To determine the concentrations of the various substances to be tested in plasma, we used the following methods and reagents: color reaction with glucose oxidase for measuring glucose using the glucose CII-test (Wako, Osaka, Japan), color reaction with *o*-cresolphthalein complexone for measuring calcium using a calcium C-test (Wako) [33], ELISA for measuring mIL-6 (Pierce Endogen systems, Rockford, IL, USA), and IRMA for measuring PTHrP (Mitsubishi Kagaku BCL, Tokyo, Japan). Tumor tissue samples for GeneChip® (Affymetrix, Santa Clara, CA, USA) analysis were resected and immediately processed with liquid nitrogen as fresh frozen tissues.

GeneChip® analysis

Tissues were lysed and total RNA was extracted and purified using Sepasol-RNA I (Wako) and an RNease column (Qiagen, Austin, TX, USA). Total RNA (5 μg) was reverse transcribed to cDNA using T7-(dT)24 primer. Biotin-labeled cRNA was synthesized from cDNA using a MEGAscript In Vitro Transcript Kit (Ambion, Austin, TX, USA). cRNA was fragmented to an average size of 50–100 nucleotides by incubation at 95°C for 35 min in 40 mM Tris–acetate (pH 8.1) containing 100 mM potassium acetate and 30 mM magnesium acetate, and then hybridized to murine Mu450A GeneChips (Affymetrix). The hybridized cRNA probes were stained with streptavidin R-phycoerythrin Molecular Probes™ (Invitrogen, Carlsbad, CA, USA) and were scanned using a confocal scanner (Affymetrix). The scanned data obtained from the microarrays were normalized to correct for small differences in the levels of the cRNA probes and were processed for signal values using Affymetrix software (LIMS 5.0).

Statistical analysis

The Mann–Whitney *U* test was used to detect statistical differences in the parameters between the clone20-bearing mice and the clone5-bearing mice or the control group and the treatment groups ($P < 0.05$). Student's *t*-test was used to detect statistical differences in the expression levels of tumor tissue between clone20 and clone5 ($P < 0.05$). Statistical analysis was carried out with SAS Preclinical Package software (SAS Institute Inc., Tokyo, Japan).

Results

Identification of factors causing cancer cachexia

Clone20 induced extensive carcass weight loss of CD2F1 mice, whereas clone5 did not (Figs. 1, 2a). We examined the involvement of PTHrP, a cytokine causing hypercalcemia in the cachectic clone20 model, where the anti-IL-6 antibody did not suppress hypercalcemia. Circulating PTHrP was significantly elevated in mice bearing clone20, whereas that in mice bearing clone5 was only slightly elevated as compared with non-tumor-bearing mice (Fig. 2b). On the other hand, circulating IL-6 was elevated in both models (Fig. 2c). To investigate comprehensively factors causing abnormalities in mice with cachexia, the differences in the mRNA expression profiles of clone20 and clone5 in tumor tissues were analyzed using GeneChip® microarray technology. In the comparison of gene expression levels for the two sublines, nine mRNAs showed differences of a ratio greater than 20; of these genes, only PTHrP was a humoral factor that would cause systemic effects in mice. (Table 1)

Administration of anti-PTHrP antibody or anti-IL-6 antibody to clone20-bearing mice

To confirm the causative roles of PTHrP and IL-6 in cachexia, neutralizing antibodies were administered to

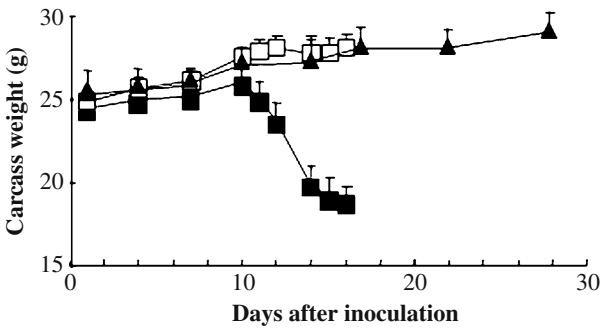

Fig. 1 Carcass weight change of mice bearing colon 26 clone5 and clone20 Carcass weight of mice (6/group) inoculated sc on day 0 with colon 26 clone20 (*closed squares*), clone5 (*closed triangles*) (1×10^6 cells/mouse), or non-tumor-bearing mice (*open squares*) (mean ± SD)

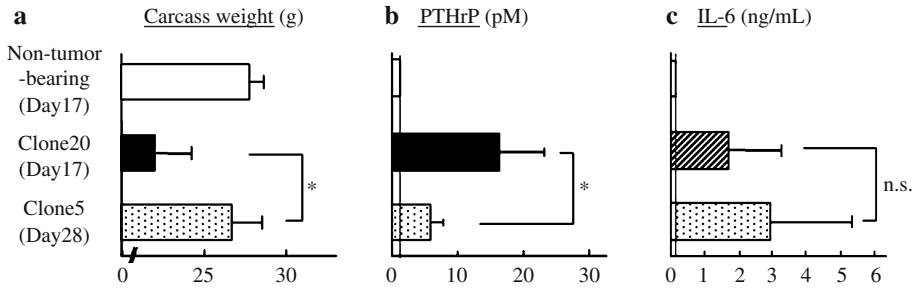

Fig. 2 Carcass weight (**a**), plasma levels of PTHrP (**b**), and IL-6 (**c**) in mice bearing colon 26 clone20, clone5. Murine IL-6 and PTHrP levels were measured by ELISA and IRMA, respectively, in the plasma of tumor-bearing or non-tumor-bearing mice on the indicated days (6 mice/group, mean ± SD). *$P < 0.05$ versus clone5 model. Non-tumor-bearing mice were age-matched with clone20-bearing mice

Table 1 Ratios of tumoral mRNA expression between clone20 and clone5

Gene name	Code	Expression ratio of mRNA clone20/clone5 (fold)
<20-fold expression		
Glutamyl-peptide cyclotransferase	1426622_a_at	50.62
PTHrP	1422324_a_at	49.92
Mus musculus Gem GTPase	1426063_a_at	28.23
Crystallin, alpha 2	1416455_a_at	27.01
Immunoglobulin kappa chain variable 28	1427455_x_at	25.62
PTHrP	1427527_a_at	25.30
Immunoglobulin kappa chain variable 28	1452417_x_at	24.91
Stimulated by retinoic acid gene	1422723_at	24.39
MMP10	1420450_at	21.76

Tumor tissues of clone20 and clone5 were collected and frozen immediately with liquid nitrogen

Details of the methods for measuring expression levels using GeneChip® arrays are described in Materials and methods. Ratio (fold) was calculated by the expression level of clone20 to that of clone5. ($N = 3$)

mice bearing clone20. Anti-PTHrP or anti-IL-6 antibody was administered to the mice throughout the weight loss period of non-treatment mice. As shown in Table 2, anti-PTHrP antibody or anti-mIL-6 antibody attenuated the wasting in spite of tumor growth enhancement. The anti-PTHrP antibody prevented the induction of hypercalcemia but suppressed neither hypoglycemia nor elevation of IL-6 (Table 2). In contrast, the anti-mIL-6 antibody prevented hypoglycemia but not hypercalcemia or elevation of PTHrP

Table 2 Suppression of cachexia induction by mIL-6 antibody or PTHrP antibody in colon 26 clone20 model

Parameters	Human IgG	Anti-mIL-6 antibody	Anti-rPTHrP antibody	Non-tumor-bearing
Number of mice per group	8	10	10	10
Tumor weight (mg)	304 ± 32	498 ± 75*	594 ± 67*	–
Carcass weight (g)	18.5 ± 1.2	20.7 ± 1.9*	23.5 ± 1.3*	28.4 ± 0.8*
Gastrocnemius weight (mg)	134 ± 6	165 ± 13*	165 ± 12*	205 ± 13*
Adipose tissue weight (mg)	17 ± 3	66 ± 68*	81 ± 37*	302 ± 62*
In plasma				
Glucose (mg/dl)	92 ± 22	142 ± 17*	118 ± 18	201 ± 29*
Calcium (mg/dl)	14.2 ± 1.6	15.6 ± 2.5	11.0 ± 0.8*	9.5 ± 0.5*
IL-6 (ng/ml)	1.53 ± 1.2	ne	2.63 ± 1.95	<0.1
PTHrP (pM)	11.5 ± 4.7	12.4 ± 5.0	ne	<1.1

Groups of ten mice were inoculated sc with 1×10^6 cells of colon 26 clone20 on day 0. On days 10 and 14 after the inoculation, the mice were administered ip either mIL-6 antibody (1 mg/mouse), PTHrP antibody (30 μg/mouse), or human IgG (1 mg/mouse). Samples were collected on day 17. Carcass weight was calculated by subtracting tumor weight from the whole body weight

ne Not examined

* $P < 0.05$ versus human IgG treatment group

(Table 2). To determine the role of hypercalcemia in the development of cachexia, ibandronate, a bisphosphonate for treating hypercalcemia, was administered. Although it suppressed hypercalcemia (control, 15.0 ± 2.7 mg/dl; ibandronate, 12.4 ± 1.7 mg/dl), it did not suppress carcass weight loss (control, 21.4 ± 3.3 g; ibandronate, 19.8 ± 2.0 g) (data not shown).

Anti-cachectic activity of capecitabine in colon 26 clone20 and Y cancer cachexia models

Capecitabine was administered to mice bearing clone20 after exhibiting body weight wasting. Carcass weight loss (Figs. 3a, 4b), adipose tissue wasting (Fig. 4c), gastrocnemius muscle wasting (Fig. 4d), hypoglycemia (Fig. 4e), and hypercalcemia (Fig. 4f) were significantly improved by capecitabine as well as by 5′-dFUrd, even at doses of capecitabine that did not reduce tumor volume (Figs. 3b, 4a). The levels of circulating IL-6 (Fig. 4g) and PTHrP (Fig. 4h) were significantly reduced by capecitabine treatment. In another cachexia model, human cervical cancer Y, capecitabine also improved body wasting, hypoglycemia, and hypercalcemia and suppressed the plasma levels of IL-6 and PTHrP (Table 3).

Discussion

Elucidation of the mechanism of cachexia induction will help in the search for potential therapeutic interventions, which possibly improve the quality of life, survival, and response to chemotherapy of advanced cancer patients. In studies on the mechanism of cachexia induction, several cytokines have been proposed as inducers in different cachexia models, such as tumor necrosis factor (TNF), [23, 26, 36], IL-1α [11, 18], interferon (IFN)-γ [16, 17], leukemia inhibitory factor [20, 31], IL-6 [8, 22, 27, 30, 39], transforming growth factor (TGF)-β [40], PIF [13], and PTHrP [14, 25]. Previously, we reported that murine colon 26 induced cancer cachexia, and that IL-6 was an essential but not the only factor responsible for inducing cachexia by demonstrating the anticachectic effect of IL-6 neutralizing antibody and comparing the serum levels of IL-6 in cachectic and non-cachectic colon 26 sublines [8]. Similar results that IL-6 is not the sole factor responsible for inducing cachexia were reported using IL-6 transgenic mice [37]. In the previous study, IL-6 neutralizing antibody did not suppress hypercalcemia in the clone20 cachectic subline [8]. In this study, we tried to clarify the involvement of PTHrP, a cytokine that causes hypercalcemia [29] and cachexia [14, 25].

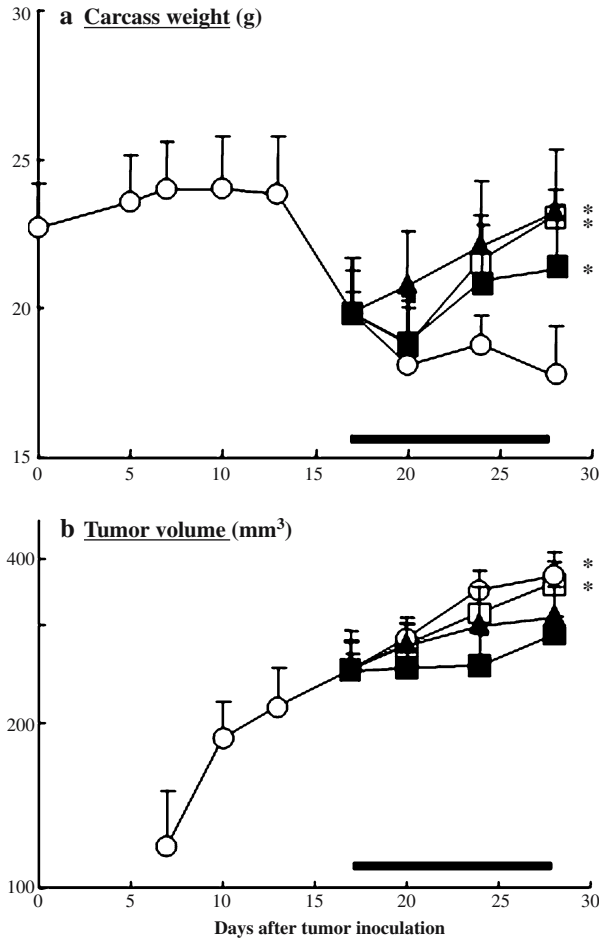

Fig. 3 Carcass weight and tumor growth treated with capecitabine and 5′-dFUrd in colon 26 clone20 model. Mice (6/group) were inoculated sc on day 0 with colon 26 clone20 (1×10^6 cells/mouse). At 17 days after the inoculation, mice were allocated to control (*open circles*) and treatment groups and were treated with 180 mg/kg capecitabine (*open squares*), 359 mg/kg capecitabine (*closed squares*) or 123 mg/kg 5′-dFUrd (*closed triangles*) for 10 days. Body weight and tumor volume were measured periodically, and carcass weight was calculated as the difference between whole body weight and estimated tumor weight (mean ± SD) $^*P < 0.05$ versus tumor-bearing controls on day 17

We found that elevated levels of circulating PTHrP protein were observed in the cachectic subline clone20 but not in clone5, and that PTHrP neutralizing antibody suppressed the wasting of mice, which implicated the involvement of PTHrP in the cachexia of mice bearing clone20. The results from GeneChip® analysis of tumor tissues of clone20 and clone5 also show the possibility of a strong involvement of PTHrP. There was no difference between the expression levels of TNF, IL-1α, IFN-γ, or TGF-β of clone20 and clone5. Either anti-PTHrP antibody or anti-mIL-6 antibody attenuated the wasting, but the suppressed parameters were not the same. The anti-PTHrP antibody prevented the induction of hypercalcemia but did not suppress

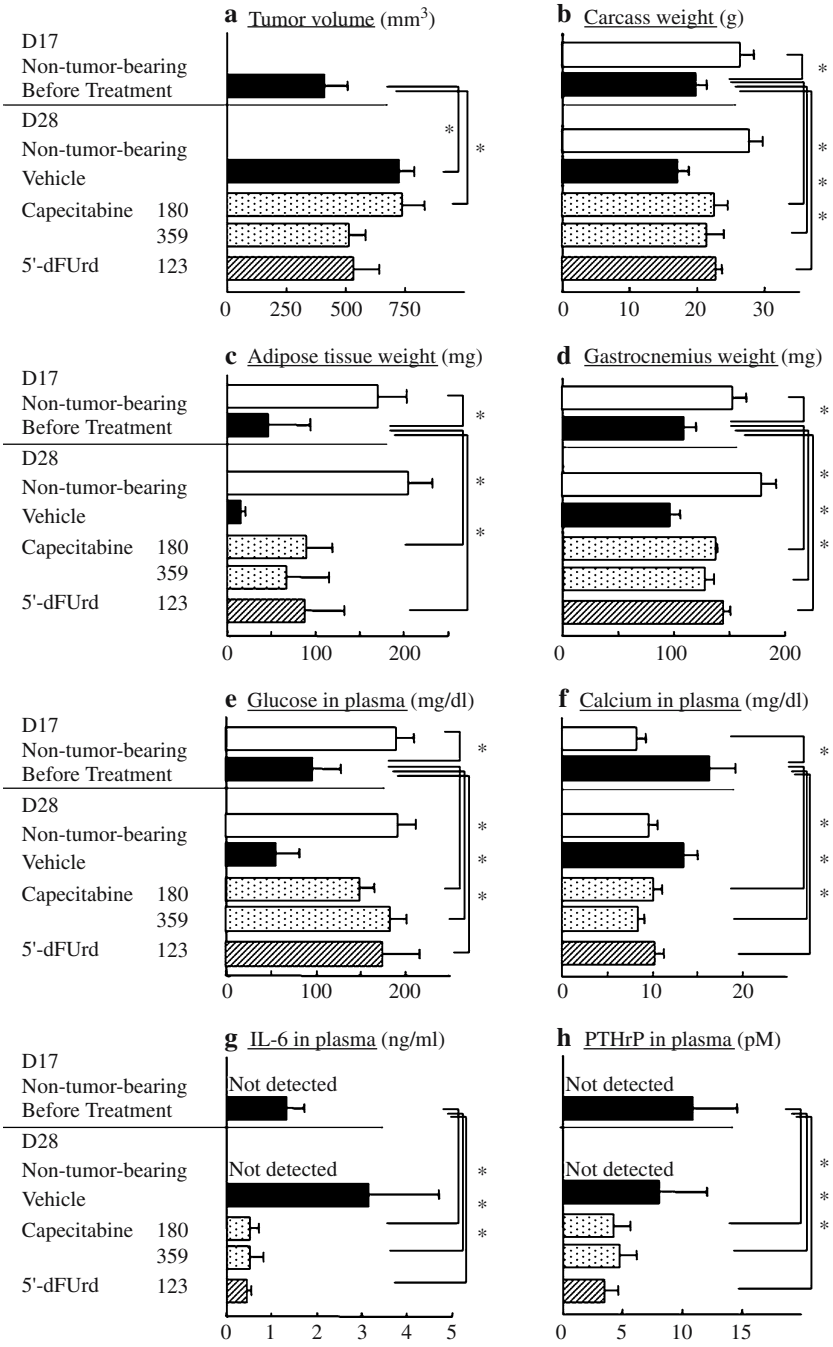

Fig. 4 Improvement of cachexia parameters by capecitabine and 5′-dFUrd in colon 26 clone20 model. Tumor volume (**a**), carcass weight (**b**), adipose tissue weight (**c**), gastrocnemius weight (**d**), plasma levels of glucose (**e**), calcium (**f**), IL-6 (**g**), and PTHrP (**h**), in colon 26 clone20 bearing mice treated with capecitabine or 5′-dFUrd (mg/kg, six mice/group). Samples were collected on day 17 when the treatment started, and on day 28, the day after the last treatment (mean ± SD). *$P < 0.05$ versus tumor-bearing controls on day 17

hypoglycemia or the elevation of IL-6, whereas the anti-IL-6 antibody prevented hypoglycemia but not hypercalcemia or the elevation of PTHrP levels. These results show that PTHrP and IL-6 were independent causative factors in this model.

Anti-cachectic effects in colon 26 have been reported for cox-2 inhibitors [2], IL-12 [19], and anti-inflammatory agents [8, 32], IL-6 blockers [7, 28], as well as IL-6 antibodies [8, 27] or IL-6R antibody [9]. In the present report, PTHrP antibody as well as IL-6 antibody prevent cachexia development. The tumor burden of antibody-treated groups became larger than that of the control group. Prevention of cachexia development could be attributed to this growth enhancement, because nutritional improvement in host organs caused by the attenuation of cachexia would also affect the tumor tissues. This does not exclude the possibility of applications in a clinical setting using the antibodies of IL-6 or PTHrP, however, because the attenuation of cachexia would open possibilities of other chemotherapeutic drug treatments and of suppressing poor responses to chemotherapy. Furthermore, cachexia

Table 3 Improvement of cachexia parameters from capecitabine in Y model

Parameters	Non-tumor-bearing	Control (day 49)	Vehicle (day 57)	Capecitabine (day 57)
Number of mice per group	6	6	6	6
Tumor volume (cm^3)	–	2.82 ± 0.64	4.55 ± 2.34	3.08 ± 1.32
Carcass weight (g)	22.1 ± 0.9	16.8 ± 1.2	15.8 ± 1.5	17.9 ± 1.4*
Plasma				
Glucose (mg/dl)	127 ± 17	59 ± 22	42 ± 41	99 ± 30*
Calcium (mg/dl)	9.8 ± 1.0	14.3 ± 2.3	14.3 ± 1.9	11.1 ± 0.6*
IL-6 (ng/ml)	<0.1	0.49 ± 0.13	0.68 ± 0.41	0.08 ± 0.03*
PTHrP (pM)	<1.1	20.1 ± 6.5	9.46 ± 1.86	2.45 ± 0.11*

Groups of six mice were inoculated sc with 8-mm^3 of Y tumor tissues on day 0. Capecitabine was administered to the mice po from day 49 to day 57 after the inoculation. Samples were collected on day 49 or day 57. Carcass weight was calculated by subtracting tumor weight from the whole body weight

* $P < 0.05$ versus control group on day 49

attenuation would benefit the QOL of patients or even prolong the survival of those bearing larger tumor burdens. Tanaka et al. [32] reported that indomethacin enhanced tumor growth concurrently with improvement of cachexia in the colon 26 model. In their report, indomethacin prolonged the survival period compared with that of the control, despite tumor growth enhancement. The increase of survival time is thought to be accompanied by an improvement in the cachectic condition, but it is not accompanied by the inhibition of tumor growth.

In the present report, we demonstrated the anti-cachectic activity of capecitabine, a derivative of 5′-dFUrd. Capecitabine (Xeloda®) is an oral fluoropyrimidine, activated at the tumor site by thymidine phosphorylase, preferentially expressed in tumor tissue. It is widely used clinically for the treatment of breast and colorectal cancers [1, 38]. Capecitabine improved body wasting, gastrocnemius/adipose tissue wasting, hypoglycemia, and hypercalcemia, even at doses too low to reduce the tumor volume in the colon 26 subline clone20 model. In addition, capecitabine also improved cachexia in the Y model, where IL-6 was reported to be involved in the development of cachexia [30]. The circulating levels of IL-6 and PTHrP were significantly reduced by capecitabine treatment, and thus it is possible that capecitabine improves cachexia by reducing levels of both IL-6 and PTHrP. We previously determined that 5′-dFUrd was able to improve murine colon 26 carcinoma, even at a low dose with no reduction in the tumor burden [5, 34]. We also examined the anticachectic activity of other chemotherapeutic agents to improve the wasting and confirmed that only 5′-dFUrd immediately improved cachexia even at doses allowing tumor growth in the cachexia model with the parent line of colon 26. Nimustine, cyclophosphamide, and 2′-deoxy-5-fluorouridine were only slightly effective in improving the wasting, whereas 5-fluorouracil, tegafur, mitomycin C, *cis*-platinum, and doxorubicin were not effective [34].

The mechanisms responsible for the reduction of the levels of IL-6 and PTHrP have not been clarified. Thioguanidine was reported to reduce PTHrP production without showing antiproliferative activity in human breast cancer MDA-MB-231 [10], although neither 5′-dFUrd nor 5-fluorouracil (5-FUra) suppressed PTHrP production of clone20 cells in vitro at non-antiproliferative concentrations (data not shown). A possible mechanism is that capecitabine attacks cytokine-overexpressing tumor cells selectively. Inflammatory cytokines are well known to induce IL-6 or PTHrP. These cytokines were also reported to induce uridine phosphorylase (UP), an enzyme that converts 5′-dFUrd to 5-FUra in colon 26 [6]. These inflammatory cytokines are thought to be abundant and induce UP in some populations of tumor cells. Therefore, there is the possibility of cells existing in tumor tissues that co-express UP and IL-6 or PTHrP. Such cells would be more susceptible to damage from capecitabine, resulting in a decrease of IL-6 and PTHrP, because capecitabine is activated by UP in these cells. Another possible mechanism is that capecitabine exerts a host-mediated effect. In colon 26, IL-6 production was reported to vary according to the inoculation site [15], indicating that the local environment might affect the cytokine production of colon 26. In this report, the colon26 tumor grown in the liver showed neither wasting nor IL-6 production in plasma and tumor tissues, whereas the colon26 inoculated subcutaneously showed both effects. Histological studies of the same colon26 in different sites revealed differences in tumor composition. Thus, the environment where tumor cells grow would be a critical factor in determining the cachectic phenotype of cancer cells. Capecitabine might have an effect on host cells to change the microenvironment in tumor tissues, which would result in improved cachexia. Further studies are required to

clarify the mechanism by which capecitabine suppresses IL-6 and PTHrP.

PTHrP has been demonstrated to be involved in bone metastasis. Capecitabine reportedly showed potent antitumor activity against bone metastasis at doses lower than those needed to reduce the primary tumor burden in a 4T1 mouse breast cancer model [12]. Blum et al. [1] reported that 47% of patients with pain achieved a significant decrease in pain intensity in a phase II study of capecitabine for metastatic breast cancer. Capecitabine might be effective against bone metastasis by reducing PTHrP. For cancer cachexia, however, there is no clinical evidence for the effect of capecitabine. In a future clinical study for cancer cachexia, however, analysis of wasting, hypoglycemia, hypercalcemia, circulating IL-6, and circulating PTHrP would be useful for evaluating cachexic conditions as well as performance status. The potential of capecitabine to improve cancer cachexia or to reduce bone metastasis warrants further examination in the clinic.

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