ANTICANCER RESEARCH 38: 4667-4676 (2018)
doi:10.21873/anticanres.12772

Investigation into Enhancing Capecitabine Efficacy in Colorectal Cancer by Inhibiting Focal Adhesion Kinase Signaling

JAE JUN SIM[1*], MIN HEE PARK[2], JEONG-HEUM BAEK[3], HAEJUN LEE[4], KEUN-YEONG JEONG[2*] and HWAN MOOK KIM[1]

[1]*Gachon Institute of Pharmaceutical Science, Gachon University, Incheon, Republic of Korea;*
[2]*MetiMedi Pharmaceuticals Co., R&D Division, Incheon, Republic of Korea;*
[3]*Division of Colon and Rectal Surgery, Department of Surgery, and* [4]*Department of Nuclear Medicine, Gil Medical Center, Gachon University College of Medicine, Incheon, Republic of Korea*

Abstract. *Background/Aim: Capecitabine is a pro-drug of 5-fluorouracil (5-FU), and is an orally available chemotherapeutic used to treat colorectal cancer (CRC). Recently, research has focused on improving its efficacy at lower doses in order to minimize its well-known toxicities. In this study, we investigated the possibility of improving the antitumor effect of capecitabine against CRC by destabilizing focal adhesion kinase (FAK) signaling. Materials and Methods: Optimal dosages for capecitabine and lactate calcium salt (LCS) were determined using the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide MTT assay. The viability of CRC cells was investigated by MTT and clonogenic assays after single or combination treatment with capecitabine and LCS. Western blot analyses were used to determine changes in the expression of components of the FAK and AKT signaling cascade, and this information was used to elucidate the underlying mechanism. A xenograft model was established to evaluate the antitumor efficacy of the combination treatment, as well as its necrotic effect and organ toxicity. Results: The addition of LCS to capecitabine treatment led to an increase in the proteolysis of the FAK signaling cascade components, including SRC proto-oncogene, non-receptor tyrosine kinase; AKT serine/threonine kinase 1; and nuclear factor-kappa B, resulting in a decrease in the viability and clonogenic ability of CRC cells. In vivo antitumor efficacy, including tumor necrosis, was significantly increased with the combination treatment relative to both single treatments, and no organ toxicity was found in any experimental group. Conclusion: The addition of LCS increased the anticancer efficacy of capecitabine at a lower dose than is currently used in human patients.*

*These Authors contributed equally to this study.

Correspondence to: Professor Hwan Mook Kim, Ph.D., Gachon Institute of Pharmaceutical Sciences, Gachon University, 191 Hambangmoe-ro, Yeonsu-Gu, Incheon, 21936, Republic of Korea. Tel: +82 328996446, Fax: +82 328204829, e-mail: hwanmook@gachon.ac.kr and Keun-Yeong Jeong, Ph.D., Head of Research and Development Division, MetiMedi Pharmaceuticals Co., R&D Division, 263 Central-ro, Yeonsu-Gu, Incheon, 22006, Republic of Korea. Tel: +82 322050541, Fax: +82 322050542, e-mail: alvirus@naver.com

Key Words: Colorectal cancer, capecitabine, lactate salt, focal adhesion kinase, toxicity, combination therapy.

Capecitabine was designed as a pro-drug of 5-fluorouracil (5-FU) with the intention of avoiding the inconvenience of intravenous administration, as is necessary for treatment with 5-FU itself (1). Capecitabine was the first orally available fluoropyrimidine approved for use in patients with breast cancer and metastatic colorectal cancer, based on significant improvements seen in clinical data (2). However, when capecitabine is used as part of a second-line regimen with oxaliplatin, irinotecan, with/without docetaxel, significant toxicities occur, such as grade 3/4 neutropenia, hand–foot syndrome, diarrhea, and sensory neuropathy (3, 4). Therefore, there is a crucial need for alternative treatment regimens that use a lower concentration of these chemotherapeutics.

Many studies have reported that dietary calcium supplementation plays a key role in the prevention of colon carcinogenesis (5, 6). Recently, there have been several studies investigating the mechanism of the calcium-mediated antitumor effect in colorectal cancer (CRC) (7-9). In previous studies, calcium was shown to increase the antitumor efficacy of 5-FU through cleavage of focal adhesion kinase (FAK) by activated calcium-dependent calpains (8, 9). Since capecitabine exhibits the same antitumor mechanisms of action as 5-FU, it may be possible to increase its efficacy by sharing the corresponding antitumor mechanism *via* calcium.

FAK, a protein kinase involved in cellular adhesion, is activated in several types of advanced-stage cancer. FAK plays an important role in cell spreading, migration, motility, apoptosis, and survival, and is a key regulator of these cellular signaling cascades (10). In CRC cells, FAK phosphorylation occurs on multiple residues, and FAK overexpression is correlated with an increase in the levels of phosphatidylinositol 3-kinase (PI3K), AKT serine/threonine kinase 1 (AKT), and nuclear factor-kappa B (NF-κB), all of which are related to cancer progression (11).

Lactate calcium salt (LCS) is a crystalline salt that is formed by the reaction of lactic acid and calcium carbonate. LCS is most commonly used in medicine to treat calcium deficiencies such as hypocalcemia syndromes. In our previous study, FAK proteolysis was induced in CRC cells by a continuous supply of LCS, and their metastatic features declined significantly (8).

In the present study, LCS was used to facilitate the diffusion of calcium into CRC cells. Next, we investigated the antitumor effect of LCS with a focus on FAK stabilization at the protein level and examined whether the addition of LCS would increase the antitumor efficacy of capecitabine against CRC.

Materials and Methods

Cell culture and reagents. Human colon cancer cell lines, HCT-116 and HT-29, were purchased from the American Type Culture Collection (Manassas, VA, USA). The cell lines were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum (Life Technologies, Gaithersburg, MD, USA), 100 IU/ml penicillin (Welgene, Daegu, South Korea), and 100 μg/ml streptomycin (Welgene). Capecitabine and LCS were purchased from Sigma-Aldrich (St. Louis, MO, USA). Primary and secondary anti-rabbit antibodies for western blot analysis were purchased from Cell Signaling Technology (Danvers, MA, USA).

Cell viability assay. HCT-116 and HT-29 cells were seeded at a density of 5,000 cells/well in 96-well plates and treated with LCS (0.5, 1, 2.5, and 5 mM), capecitabine (1, 5, 10, and 20 μM), or a combination of the two (2.5 mM LCS and 20 μM capecitabine) for 48 h. Cell viability assay was then performed using 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, 5 mg/ml).

Colony formation assay. CRC cells were seeded at a density of 2.5×10^2 cells/well in a 6-well plate. After 24 h, the medium was changed, and the cells were treated with 2.5 mM LCS, 20 μM capecitabine, or a combination of the two. After 10 days, the colonies were fixed with methanol and stained using hematoxylin (Thermo Fisher Scientific, Waltham, MA, USA). The stained colonies were counted under an optical microscope (Olympus, Center Valley, PA, USA).

Western blot analysis. Whole treated and untreated CRC cells were lysed with RIPA buffer (Thermo Fisher Scientific). The resulting protein extracts (20 μg) were loaded onto 10% polyacrylamide gels and were separated by sodium dodecyl sulfate polyacrylamide gel electrophoresis (Bio-Rad, Hercules, CA, USA), and the separated proteins were then transferred onto polyvinylidene fluoride membranes (Merck Millipore, Billerica, MA, USA). After blocking in 5% skim milk (Bio-Rad, Hercules, CA, USA) for 1 h, membranes were incubated overnight at 4˚C with primary antibody diluted in tris-buffered saline/polysorbate 20 (TBST) containing 0.5% tween-20 (Sigma-Aldrich). The specific dilutions for each antibody were as follows: FAK, 1:1000; SRC proto-oncogene, non-receptor tyrosine kinase (SRC), 1:1000; AKT, 1:1000; NF-κB, 1:1000; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH), 1:5000. membranes were then washed with TBST and incubated for 2 h with anti-rabbit secondary antibody (1:5000). Immunoblots were exposed to x-ray film (Agfa, Leverkusen, Germany) according to the manufacturer's protocol. Qualitative analysis was then performed.

Xenograft animal model. All experiments were performed under the institutional guidelines established by the Institutional Animal Care and Use Committee at Gachon University (Approval #: LCDI-2017-0090). Eight-week-old male BALB/c nude mice were purchased from Orientbio (Seong-Nam, South Korea). All animals were maintained in a 12-h light/dark cycle at 22-25˚C with free access to food and water. HT-29 cells (1×10^7 cells) were injected into the dorsal flank to investigate the antitumor efficacy of capecitabine (60 mg/kg, *per os*), LCS (2 mg/kg, subcutaneously), or a combination of the two. Three mice per group were used for the single and combination treatments while five mice were assigned for the control group. Capecitabine was administered five times a week for 2 weeks, and LCS was administered daily for 21 days. Measurements were taken of the tumor size three times per week and of body weight every week, and tumor volumes were calculated. Twenty-nine days after tumor cell injection, tumors were isolated for weighing or staining with hematoxylin and eosin (H&E). To confirm the toxicity, post-mortem screenings were also conducted.

Statistical analysis. All data are presented as the mean±standard deviation (SD). Statistical significance was analyzed using Student's *t*-test depending on the normality of the data. Values of $p<0.05$ were considered to be statistically significant (Sigmastat ver. 3.5; Systat Software Inc., Chicago, IL, USA).

Results

Increasing LCS and capecitabine doses gradually reduced the survival of CRC cells. In order to select optimal concentrations of LCS and capecitabine for the study, the viability of CRC cells (HCT-116 and HT-29) was investigated following treatment with different doses of LCS and capecitabine up to 5 mM and 20 μM, respectively (Figure 1). Concentrations of LCS between 1 and 5 mM significantly reduced the viability of CRC cells. Although the viability of CRC cells was significantly lowered after treatment with 5 mM LCS, the concentration of 2.5 mM was selected for subsequent study. HCT-116 and HT-29 cell viability decreased to 67.42±7.14% and 71.63±3.08%, respectively, after treatment with 2.5 mM LCS (Figure 1A). Only capecitabine doses of 10 and 20 μM significantly reduced CRC cell viability; the concentration of 20 μM was

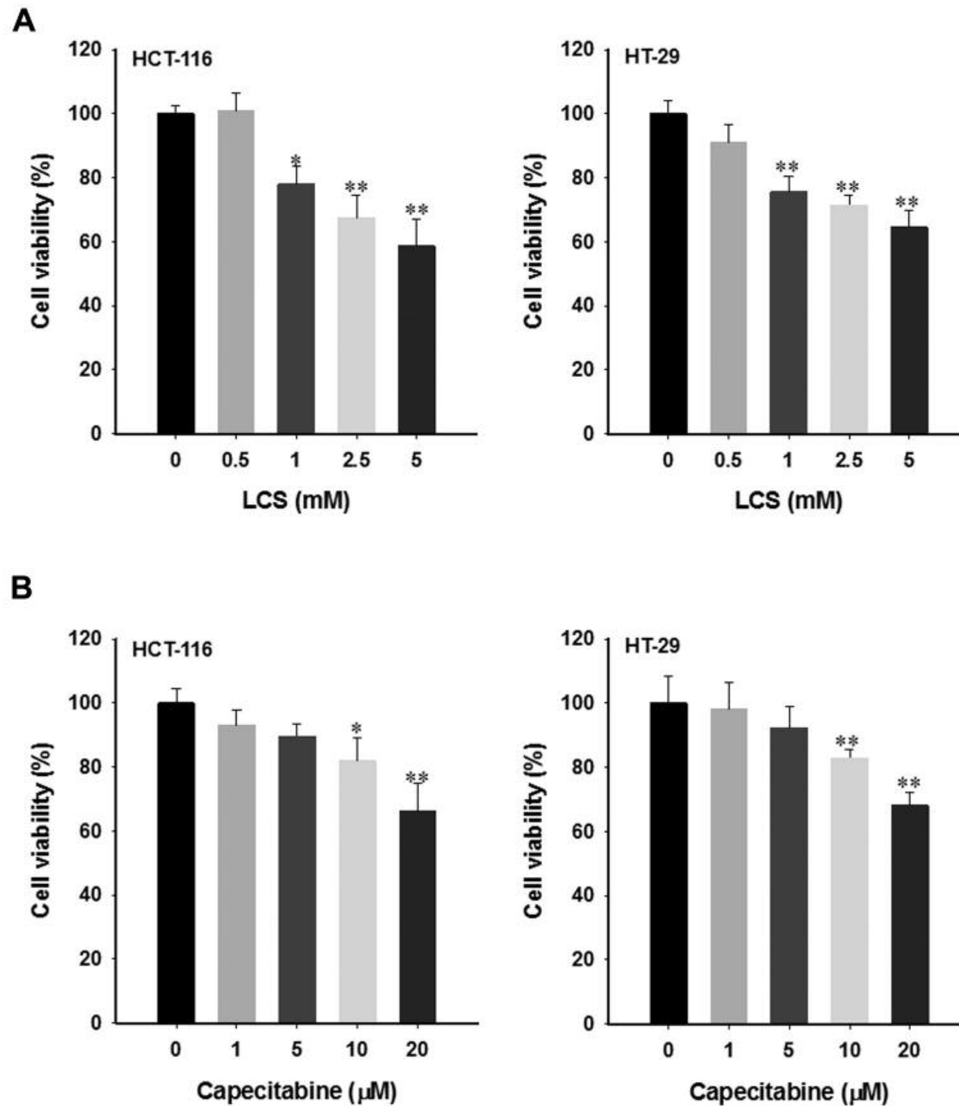

Figure 1. *Quantitative analysis of the viability of HCT-116 and HT-29 colorectal cancer cells following different doses of A: LCS and B: capecitabine treatment. The experiments were performed in quintuplicate. Significantly different at: *p<0.05 and **p<0.001 vs. control group. Results are the mean±SD.*

selected for the study. CRC cell viability significantly decreased to 66.72±9.47% and 68.19±3.94% in HCT-116 and HT-29, respectively, with 20 μM capecitabine treatment (Figure 1B).

The combination of LCS and capecitabine further reduced the viability of CRC cells. CRC cells were treated with 2.5 mM LCS and 20 μM capecitabine individually and combined (Figure 2). Representative images of HT-29 and HCT-116 morphology are shown in Figure 2A and C. The combination treatment of LCS and capecitabine resulted in a smaller number of viable cells than did the single treatments, and the viable cells were of poor condition (Figure 2A and C). The viability of HCT-116 cells was more than halved with the combination treatment compared with single treatment of LCS or capecitabine (Figure 2B). In HT-29 cells, viability was almost half that after single treatment with LCS or capecitabine (Figure 2D).

The combination of LCS and capecitabine reduced the clonogenicity of CRC cells. Clonogenicity was examined to compare the effect of long-term single and combination treatment of LCS and capecitabine on CRC cell colony formation. Representative images of HCT-116 and HT-29

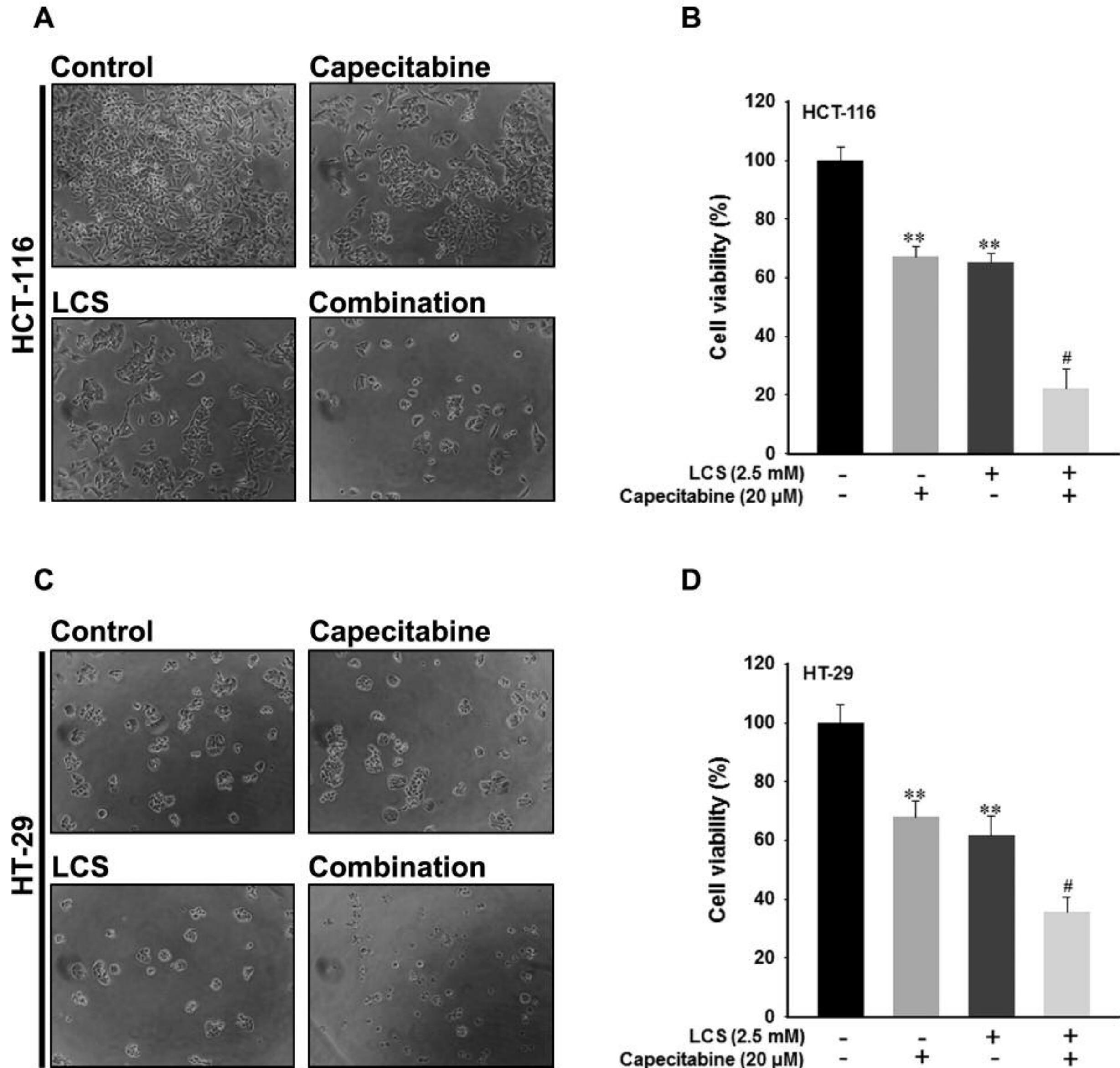

Figure 2. *Measurement of cell viability to confirm the short-term antitumor effect of treatment of HCT-116 (A, B) and HT-29 (C, D) colorectal cancer cells with lactate calcium salt (LCS) and capecitabine, alone and in combination. A and C: Representative images showing the morphology of colorectal cancer cells after treatment. B and D: Quantitative analysis of cell viability. The experiments were performed in quintuplicate. Results are the mean±SD. Significantly different at: *$p<0.05$ and **$p<0.001$ vs. control, and $^{\#}p<0.05$ vs. all other groups. Combination: Capecitabine + LCS.*

colonies are shown in Figure 3A and C. The combination treatment of LCS and capecitabine resulted in smaller colony sizes than the single treatments (Figure 3A and C). The combination treatment also led to a significant reduction in the number of HCT-116 and HT-29 colonies compared with capecitabine and LCS alone (Figure 3B and D).

LCS targets the AKT signaling cascade via FAK destabilization. To investigate the underlying mechanism of the combination effect, the expression of FAK and AKT signaling cascade molecules, and SRC and NF-κB, were examined at the protein level (Figure 4). Treatment with capecitabine alone did not affect the expression of FAK in CRC cells (Figure 4A). In HCT-116 cells, SRC expression decreased after treatment with capecitabine alone despite no change in FAK expression (Figure 4A); slight decreases in the expressions of AKT and NF-κB were also observed (Figure 4). However, treatment with capecitabine alone did not appear to affect the

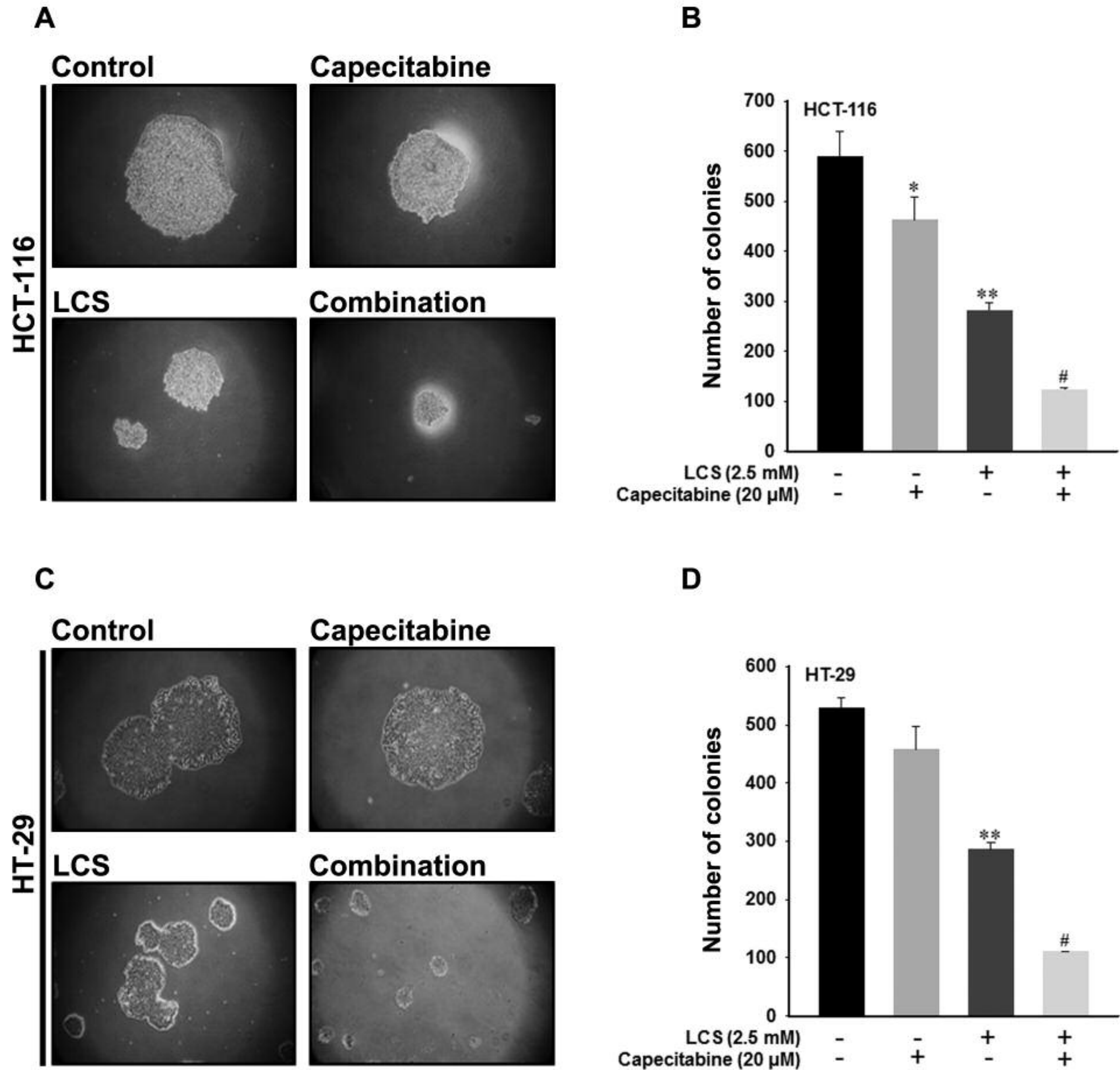

Figure 3. *Measurement of clonogenic ability to confirm the long-term antitumor effect of treatment of HCT-116 and HT-29 colorectal cancer cells with lactate calcium salt (LCS) and capecitabine, alone and in combination. A: Representative images of the morphology of colorectal cancer cells after treatment. B: Quantitative analysis of the number of colonies. The experiments were performed in triplicate. Results are the mean±SD. Significantly different at: *p<0.05 and **p<0.001 vs. control, and #p<0.05 vs. all other groups. Combination: Capecitabine + LCS.*

expression levels of FAK and AKT signaling cascades in HT-29 cells (Figure 4). In contrast, LCS treatment clearly reduced the expression of FAK and AKT signaling molecules in both cell lines (Figure 4A). Moreover, the results indicated that the combination treatment caused a clear decrease in FAK level and an increase in the cleaved forms of signaling molecules SRC, AKT, and NF-κB, relative to the single treatments (Figure 4B).

The combination of LCS and capecitabine enhanced the antitumor effect. In order to compare the *in vivo* efficacy of the combination treatment with the single treatments, capecitabine was orally administered five times a week for 2 weeks, or LCS was subcutaneously administered twice daily for 3 weeks, or a combination of the two was administered (Figure 5). In the capecitabine-treated group, mean tumor volume was 2,085.39±491.16 mm^3 on the final day of measurement

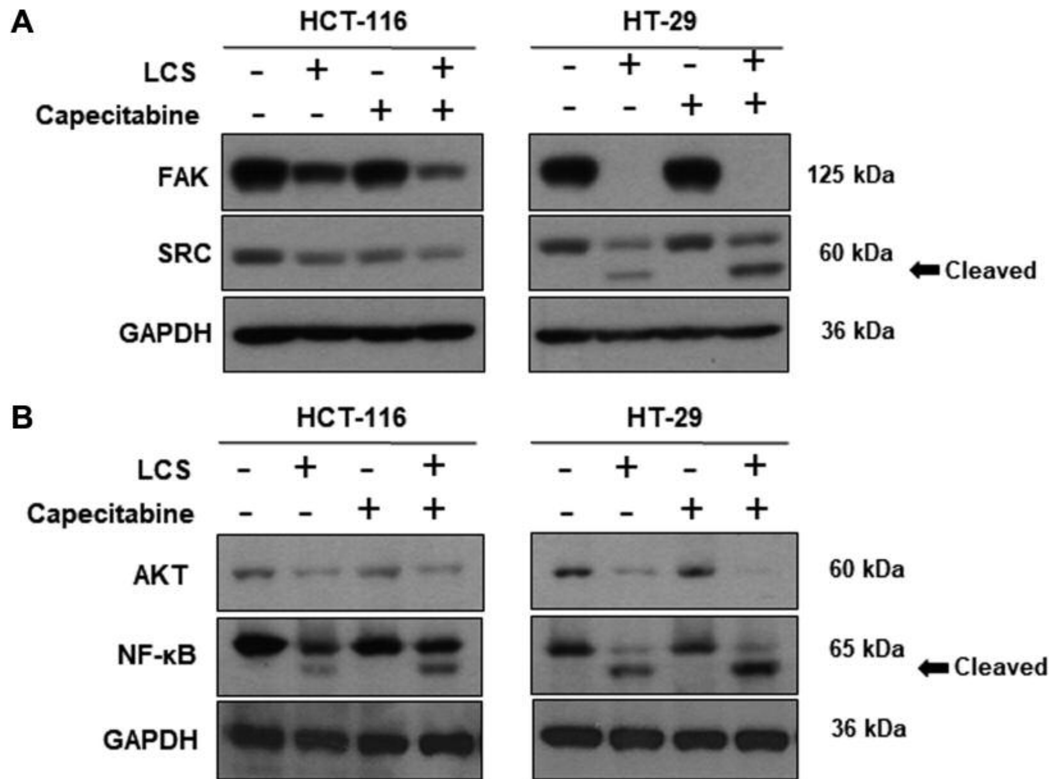

Figure 4. *Western blot analysis of HCT-116 and HT-29 colorectal cancer cells after treatment with lactate calcium salt (LCS) and capecitabine, alone and in combination, for LCS-mediated inhibition of focal adhesion kinase (FAK) signaling cascade elements. Qualitative analysis of the expression of A: FAK and SRC proto-oncogene, non-receptor tyrosine kinase (SRC), and B: AKT serine/threonine kinase 1 (AKT) and nuclear factor-kappa B (NF-κB). GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.*

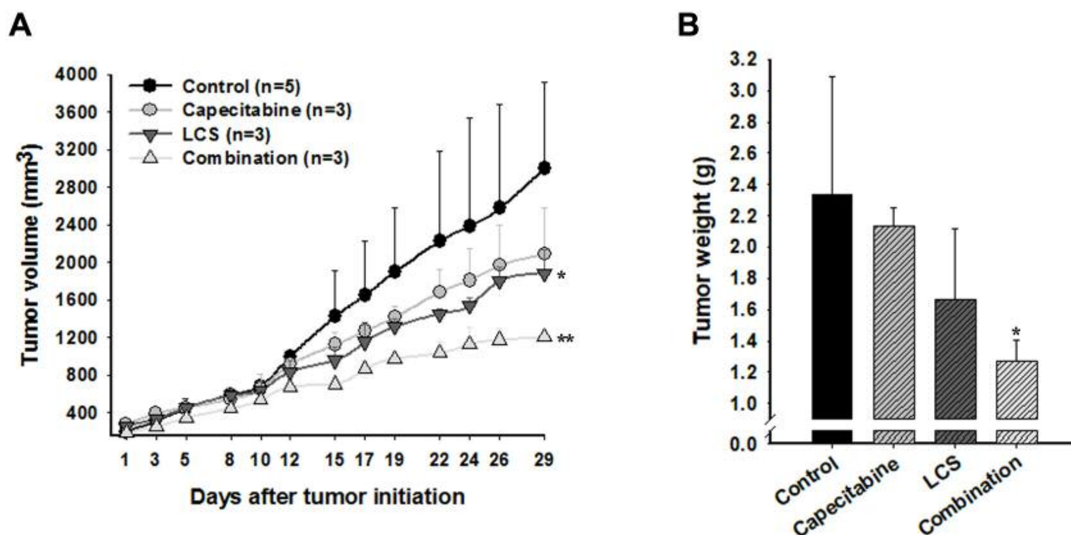

Figure 5. *In vivo antitumor effect of combined lactate calcium salt (LCS) and capecitabine treatment in a colorectal cancer xenograft model. Quantitative analysis of A: tumor volume and B: final tumor weight in mice bearing HT-29 cell-derived tumors treated with LCS and capecitabine, alone and in combination. Results are the mean±SD. Significantly different at: *p<0.05 vs. control, and **p<0.001 vs. all other groups. Combination: Capecitabine + LCS. Scale bar, main image: 100 μm, inset: 200 μm.*

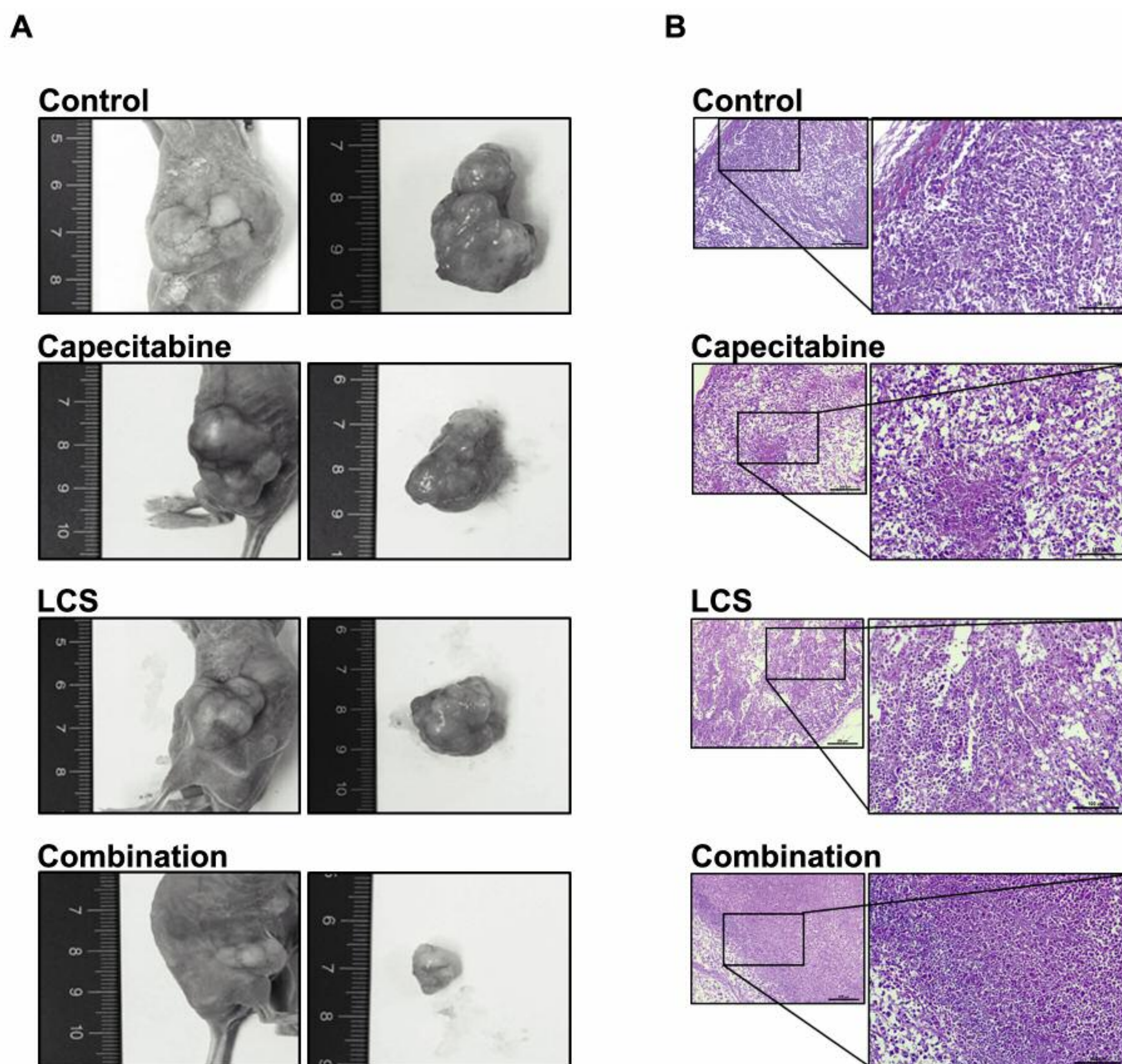

Figure 6. *Histological analysis of tumor tissues from mice bearing HT-29 cell-derived tumors treated with LCS and capecitabine, alone and in combination. A: Representative images of tumors following autopsy. B: Hematoxylin and eosin staining was used to reveal tumor cell differentiation and apoptosis. Capecitabine treatment caused partial necrosis, while LCS caused internal necrosis and combination treatment caused total necrosis, including of the superficial part. Combination: Capecitabine + LCS.*

compared to the control group (2,999.87±917.47 mm^3); however, the difference was not significant (Figure 5A). In the LCS-treated group, final tumor volume was significantly lower (1,884.19±244.65 mm^3) than that of the control (Figure 5A). The final tumor volume after the combination treatment was significantly lower at 1,201.86±42.94 mm^3 compared with the control and single treatments (Figure 5A). A significant decrease in tumor weight (1.27±0.14 g) was only observed in the combination treatment group (Figure 5B).

Representative images of the tumors are shown in Figure 6A. While aggressive growth of the tumor was observed in the control group, a reduction in tumor growth after single treatments was observed. In the combination treatment group, tumor growth was morphologically inhibited compared to the single-treated groups (Figure 6A). A histological analysis of the control group revealed high cell proliferation and a low rate of apoptosis, while partial and internal necrosis were observed after treatment with capecitabine or LCS, respectively. Following combination treatment, necrosis was observed throughout tumor mass, including the superficial part (Figure 6B). Organ toxicities were not found in any animals, and there were no significant differences in body weight between the groups (Figure 7).

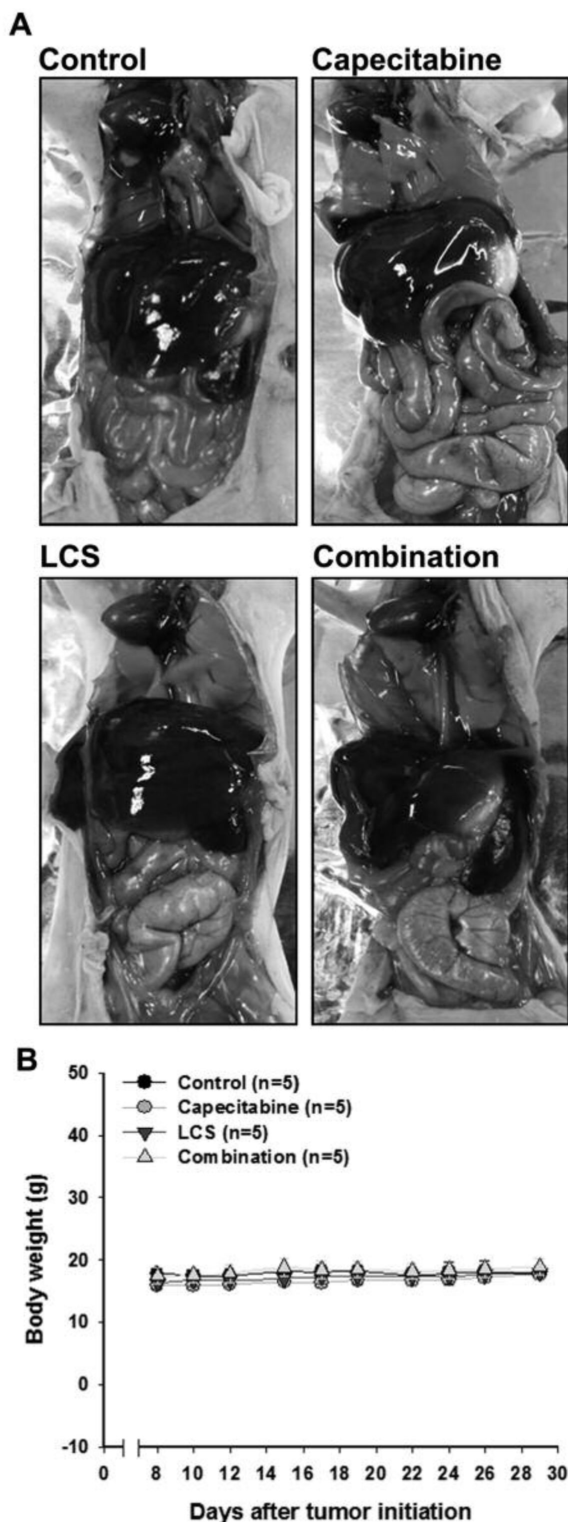

Figure 7. *Post-mortem screening to assess toxicity after treatment of mice bearing HT-29 cell-derived tumors with lactate calcium salt (LCS) and capecitabine, alone and in combination. A: Representative images for sign of organ toxicity following autopsy. B: Quantitative analysis for body weight comparison among the groups. Results are the mean±SD. Combination: Capecitabine + LCS.*

Discussion

In this study, the effect of LCS on the antitumor efficacy of capecitabine and on the destabilization of FAK and AKT signaling in CRC cells was examined. The results demonstrated that the addition of LCS enhanced the antitumor efficacy of capecitabine by inducing tumor necrosis in the whole tumor.

Capecitabine is a rationally designed 5-FU pro-drug, and is an oral fluoropyrimidine carbamate that preferentially collects in tumor tissue (12). When capecitabine is administered to patients, it is metabolized to 5-FU *via* an enzymatic cascade that is mediated by thymidine phosphorylase (8, 13). This induces a deoxynucleotide pool inequity, which is one of the factors that leads to DNA destruction (8, 14). Therefore, the inhibition of thymidylate synthase is the main mechanism of action of capecitabine, as is seen with 5-FU (12).

The rationale for the selection of 2.5 mM LCS and 20 μM capecitabine in the *in vitro* study was based on the results of CRC cell viability, as seen in the MTT assay. The optimal concentration of LCS was determined to be that causing 60–70% reduction in *in vitro* cell viability. The optimal concentration of capecitabine was selected as that which caused a similar reduction in cellular viability to that of LCS. Although the *in vitro* MTT analysis demonstrated a lower survival rate of CRC cells following treatment with even greater concentrations of LCS or capecitabine, these concentrations were chosen in order to investigate the synergistic effect of the combination treatment.

The *in vitro* antitumor efficacy of capecitabine in long-term administration (clonogenic assay) was much lower than that in short-term administration (MTT assay). This was due to the fact that in the clonogenic assay, the cancer cells formed colonies whose internal environments are hypoxic. It is a well-established theory that a hypoxic environment in tumors affects the responsiveness of cancer cells to chemotherapy and promotes cell survival and resistance to chemotherapy (15). Notwithstanding this problem, the number of colonies was significantly reduced by the combination as compared to single treatment with LCS or capecitabine. Therefore, it was confirmed that combination treatment with LCS and capecitabine is effective even in hypoxic environments that resist chemotherapy.

The FAK signaling cascade has been associated with the activation and regulation of signaling pathways that are important for the survival of cancer cells (16, 17). AKT is a part of the FAK and PI3K signaling cascades and activates the expression of NF-κB, which is a step in the survival process of cancer cells (16). The present study demonstrated that LCS caused FAK proteolysis, accompanied by a reduction in the levels of proteins participating in the FAK signaling cascade, namely SRC, AKT, and NF-κB (8). This inhibition of the signaling cascades by LCS is a different anticancer mechanism than that of capecitabine, suggesting that the combination of

LCS and capecitabine may enhance each other's antitumor effect without overlapping modes of action.

According to a standard regimen determined by the U.S Food and Drug Administration, capecitabine is administered orally to patients with CRC at a dose of 1,250 mg/m^2 twice daily for 2 weeks, equivalent to a daily dose of approximately 4.3 g/person (18). In this study, the daily dose for capecitabine was 60 mg/kg/mouse, and the human equivalent dose (HED) was calculated to be 0.292 g/person/day. This was a very much lower dose than the standard regimen of 4.3 g/person/day. Our results indicate that combination treatment with LCS enhanced the antitumor effect of low-dose capecitabine on CRC and that the addition of LCS allowed the reduction of the therapeutic dose of capecitabine required, which is clinically desirable (19).

In this study, 1 mg/kg LCS (HED: about 9.7 mg/person/day) was administered twice daily during the experiment. Previous studies have reported on the use of LCS to supply a low dose of calcium (8, 9) in patients with hypocalcemia. Despite many reports on the positive effect LCS in preventing colon carcinogenesis, controversy still exists regarding the effectiveness of calcium supplementation in CRC (20, 21). Thus, we recognize that the further research is required to overcome the controversy on calcium supplementation and its effectiveness in CRC.

Conclusion

The combination of LCS and capecitabine significantly inhibited CRC progression, even when a dose of capecitabine below the clinical standard level was used. However, before the use of LCS as part of a clinical regimen, further research is required to examine other parameters, such as maximum tolerated dose, toxicity, and the optimal route for administration.

Conflicts of Interest

There are no potential conflicts of interest to declare in regard to this study.

References

1 Aguado C, Garcia-Paredes B, Sotelo MJ, Sastre J and Diaz-Rubio E: Should capecitabine replace 5-fluorouracil in the first-line treatment of metastatic colorectal cancer? World J Gastroenterol *20*: 6092-6101, 2014.

2 Stockler MR, Harvey VJ, Francis PA, Byrne MJ, Ackland SP, Fitzharris B, Van Hazel G, Wilcken NR, Grimison PS, Nowak AK, Gainford MC, Fong A, Paksec L, Sourjina T, Zannino D, Gebski V, Simes RJ, Forbes JF and Coates AS: Capecitabine *versus* classical cyclophosphamide, methotrexate and fluorouracil as first-line chemotherapy for advanced breast cancer. J Clin Oncol *29*: 4498-4504, 2011.

3 Lee CS, Ryan EJ and Doherty GA: Gastro-intestinal toxicity of chemotherapeutics in colorectal cancer: The role of inflammation. World J Gastroenterol *20*: 3751-3761, 2014.

4 Lorenzen S, Duyster J, Lersch C, von Delius S, Hennig M, Bredenkamp R, Peschel C and Lordick F: Capecitabine plus docetaxel every 3 weeks in first-and second-line metastatic oesophageal cancer: Final results of a phase II trial. Br J Cancer *92*: 2129-2133, 2005.

5 Yang K, Lamprecht SA, Shinozaki H, Fan K, Yang W, Newmark HL, Kopelovich L, Edelmann W, Jin B, Gravaghi C, Augenlicht L, Kucherlapati R and Lipkin M: Dietary calcium and cholecalciferol modulate cyclin D1 expression, apoptosis and tumorigenesis in intestine of adenomatous polyposis coli1638N/+ mice. J Nutr *138*: 1658-1663, 2008.

6 Lamprecht SA and Lipkin M: Chemoprevention of colon cancer by calcium, vitamin D and folate: molecular mechanisms. Nat Rev Cancer *3*: 601-614, 2003.

7 Jo YK, Park MH, Choi H, Lee H, Park JM, Sim JJ, Chang C, Jeong KY and Kim HM: Enhancement of the antitumor effect of methotrexate on colorectal cancer cells *via* lactate calcium salt targeting methionine metabolism. Nutr Cancer *69*: 663-673, 2017.

8 Park M, Sundaramoorthy P, Sim JJ, Jeong KY and Kim HM: Synergistically Anti-metastatic effect of 5-flourouracil on colorectal cancer cells *via* calcium-mediated focal adhesion kinase proteolysis. Anticancer Res *37*: 103-114, 2017.

9 Jeong KY, Park M, Kim IU, Sim JJ and Kim HM: Enhancing 5-fluorouracil efficacy in a primary colorectal cancer by long-lasting calcium supplementation. Anticancer Res *37*: 2959-2964, 2017.

10 Zhang J and Hochwald SN: The role of FAK in tumor metabolism and therapy. Pharmacol Ther *142*: 154-163, 2014.

11 Chen Y, Wang Z, Chang P, Xiang L, Pan F, Li J, Jiang J, Zou L, Yang L, Bian Z and Liang H: The effect of focal adhesion kinase gene silencing on 5-fluorouracil chemosensitivity involves an Akt/NF-kappaB signaling pathway in colorectal carcinomas. Int J Cancer *127*: 195-206, 2010.

12 Schellens JH: Capecitabine. Oncologist *12*: 152-155, 2007.

13 Van Cutsem E, Hoff PM, Harper P, Bukowski RM, Cunningham D, Dufour P, Graeven U, Lokich J, Madajewicz S, Maroun JA, Marshall JL, Mitchell EP, Perez-Manga G, Rougier P, Schmiegel W, Schoelmerich J, Sobrero A and Schilsky RL: Oral capecitabine *vs.* intravenous 5-fluorouracil and leucovorin: integrated efficacy data and novel analyses from two large, randomised, phase III trials. Br J Cancer *90*: 1190-1197, 2004.

14 Longley DB, Harkin DP and Johnston PG: 5-Fluorouracil: Mechanisms of action and clinical strategies. Nat Rev Cancer *3*: 330-338, 2003.

15 Cosse JP and Michiels C: Tumour hypoxia affects the responsiveness of cancer cells to chemotherapy and promotes cancer progression. Anticancer Agents Med Chem *8*: 790-797, 2008.

16 Bouchard V, Demers MJ, Thibodeau S, Laquerre V, Fujita N, Tsuruo T, Beaulieu JF, Gauthier R, Vezina A, Villeneuve L and Vachon PH: FAK/SRC signaling in human intestinal epithelial cell survival and anoikis: differentiation state-specific uncoupling with the PI3-K/AKT-1 and MEK/ERK pathways. J Cell Physiol *212*: 717-728, 2007.

17 Sulzmaier FJ, Jean C and Schlaepfer DD: FAK in cancer: Mechanistic findings and clinical applications. Nat Rev Cancer *14*: 598-610, 2014.

18 Hirsch BR and Zafar SY: Capecitabine in the management of colorectal cancer. Cancer Manag Res *3*: 79-89, 2011.

19 Hennessy BT, Gauthier AM, Michaud LB, Hortobagyi G and Valero V: Lower dose capecitabine has a more favorable therapeutic index in metastatic breast cancer: retrospective analysis of patients treated at M.D. Anderson Cancer Center and a review of capecitabine toxicity in the literature. Ann Oncol *16*: 1289-1296, 2005.
20 Wactawski-Wende J, Kotchen JM anderson GL, Assaf AR, Brunner RL, O'Sullivan MJ, Margolis KL, Ockene JK, Phillips L, Pottern L, Prentice RL, Robbins J, Rohan TE, Sarto GE, Sharma S, Stefanick ML, Van Horn L, Wallace RB, Whitlock E, Bassford T, Beresford SA, Black HR, Bonds DE, Brzyski RG, Caan B, Chlebowski RT, Cochrane B, Garland C, Gass M, Hays J, Heiss G, Hendrix SL, Howard BV, Hsia J, Hubbell FA, Jackson RD, Johnson KC, Judd H, Kooperberg CL, Kuller LH, LaCroix AZ, Lane DS, Langer RD, Lasser NL, Lewis CE, Limacher MC, Manson JE and Women's Health Initiative I: Calcium plus vitamin D supplementation and the risk of colorectal cancer. N Engl J Med *354*: 684-696, 2006.
21 Keum N, Aune D, Greenwood DC, Ju W and Giovannucci EL: Calcium intake and colorectal cancer risk: Dose-response meta-analysis of prospective observational studies. Int J Cancer *135*: 1940-1948, 2014.

Received June 14, 2018
Revised June 26, 2018
Accepted June 28, 2018