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Original Research Article

Effect of a combination of oxaliplatin and fluoropyrimidine derivative molecule S-1 on expression levels of IL-9 and CysLTR-1 in a rat model of colon cancer

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Abstract

Purpose: *To investigate the effect of a combination of oxaliplatin and fluoropyrimidine derivative molecule S-1 on the expression levels of IL-9 and CysLTR-1 in rats with colon cancer.*
Methods: *One hundred and eighty healthy Spraque Dawley rats were randomly assigned to groups A (healthy control), B (model), and C (treatment) with 60 rats in each group. Western blotting was used to determine contents of CysLTR-1 and related pathway proteins, while the levels of IL-9 and IFN-γ were evaluated by enzyme-linked immunosorbent assay (ELISA). Cell Counting Kit-8 (CCK-8) was used to determine cell proliferation, while cell apoptosis was assessed using flow cytometry.*
Results: *Tumor volume was larger and tumor diameter was longer in group B than in group C ($p < 0.05$). The expression levels of CysLTR-1, LTD4, β-catenin, COX-2, IL-9 and IFN-γ were significantly up-regulated in groups B and C, relative to group A ($p < 0.05$). Cell proliferation was also significantly faster in groups B and C than in group A, while apoptotic rate was lower ($p < 0.05$).*
Conclusion: *Combined use of OX and S-1 inhibits CysLTR-1 expression level in colon cancer rats. Furthermore, it down-regulates IL-9 expression, thereby inhibiting the proliferation and apoptosis of tumor cells. Therefore, the combination treatment has potentials for development as a therapeutic strategy for the management of colon cancer.*

Keywords: *Oxaliplatin, Fluoropyrimidine derivative molecule, Colon cancer, CysLTR-1*

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INTRODUCTION

Colon cancer, the third most commonly diagnosed cancer in the world and the fourth most fatal cancer, has a long history in developed countries, and its incidence has increased rapidly in developing countries in recent years [1]. Rectal cancer starts from the tissues of the inner wall of the rectum or colon. The tissues grow slowly into blood or lymphatic vessels, and finally form malignant tumors on the rectum or colon wall, with a high incidence of metastasis [2]. Therefore, studies on the

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treatment of colon cancer are important aspects of clinical research.

Cysteinyl leukotrienes (CysLTs) mediate cancer cell metastasis promoted by exosomes, while cysteinyl leukotriene receptors (CysLTRs) mediate pathophysiological functions of CysLTs [3]. The expression of CysLTRs is upregulated in patients with colorectal cancer. Studies have shown that CysLTR-1 signal transduction induces the expression of β-catenin and cyclooxygenase 2 (COX-2) due to stimulation by leukotriene D4 (LTD4), leading to the proliferation, survival, and migration of cancer cells, as well as increases in inflammatory cytokine levels [4-6]. Although oxaliplatin (OX) is used for cancer treatment, its therapeutic effect is unsatisfactory. The efficacy of OX is limited by its neurotoxicity which causes great harm to patients [7]. Similarly, fluoropyrimidine derivative molecule S-1 is usually used for treating colon cancer, but patients do not benefit from this drug due to high risk of post-treatment recurrence [8]. There are no extant studies on the efficacy of combined therapy with these two drugs, as well as the effect of their combination on CysLTR-1 and inflammatory cytokines (e.g., IL-9) in colon cancer.

The present research was carried out to study the influence of OX and S-1 combination on expression levels of IL-9 and CysLTR-1 in colon cancer, and to investigate its specific effect on the disease in a rat model of colon cancer.

EXPERIMENTAL

Animals

A total of 180 healthy Spraque Dawley (SD) rats were purchased from Hunan SJA Laboratory Animal Co. Ltd. They were similar in age, body length, and body mass, and were fed in an appropriate environment (light/day cycle, temperature and humidity. The study received approval from the Ethics Committee of the Affiliated Hexian Memorial Hospital of Southern Medical University (approval no. 2018MSXM010), and was carried out in line with international guidelines for use of experimental animals [9].

Reagents and instruments

The reagents used, and their sources (in parenthesis) were: human colon cancer HCT116 cells (Fenghui Shengwu, Hunan, China); trypsin (Life technologies, USA), 0.25 % phosphate buffered solution (PBS; Thermo Fisher Scientific, China); rat CysLTR-1 enzyme-linked immunosorbent assay (ELISA) kit (Yiyan Biotechnology Co. Ltd, Shanghai, China); IL-9 ELISA kit (Yansheng Biochemical Reagent Co. Ltd, Shanghai, China); OX (SFDA approval number: H20093487, Haizheng Pharmaceutical Co Ltd, Zhejiang, China); S-1 (SFDA approval number: H20113281, Hengrui Medicine Co. Ltd, Jiangsu, China); TBST (Biolab Science and Technology Co. Ltd, Beijing, China); loading buffer (Beyotime Institute of Biotechnology), and xylene, ethanol, hematoxylin, eosin, and neutral balsam (Sigma, USA).

The instruments used were incubator (Shanghai Feiyue Experimental Instrument Co. Ltd), bovine serum albumin protein quantification kit (Beyotime Inst. Biotech), NC membrane (Amresco), full wavelength microplate reader (Hangzhou Aosheng Instrument Co. Ltd), cell cycle assay kit (Shanghai Enzyme-linked Biotechnology Co. Ltd), and CCK-8 assay kit (Shanghai Jingkang Bioengineering Co. Ltd). The apoptosis detection kit was a transfection reagent.

Animal modeling and grouping

The 180 rats were fed for 7 days before the experiment, in strict accordance with regulations on the management and use of experimental animals. They were randomly assigned to groups A, B, and C, with 60 rats/group.

Cell culture and establishment of colon cancer model

The HCT116 cells were maintained overnight at 37 ℃ in an atmosphere of 5 % carbon dioxide. When the bottom of the culture flask was covered with cells, the cells were digested with trypsin and sub-cultured. Thereafter, the colon cancer model was established when the concentration reached 1 × 10^8 cells/mL under a microscope. The colon cancer model was established by inoculating 0.1 mL of the cells under the back skin of each rat. Rats in group A (healthy control group) were not subcutaneously injected, but were intragastrically fed with distilled water (3 mL) once daily for 2 weeks. Rats in group B (model group) were subcutaneously injected with 0.1 mL of cells, and then intragastrically administered distilled water (3 mL) once daily for 2 weeks. In group C (treatment group), rats were subcutaneously injected with cells (0.1mL), and then treated with 3 mL of OX at a dose of 0.5 g/kg, in combination with S-1, once daily for 2 weeks. On the 15th day after drug treatment, the rats were sacrificed. Tumor tissues were taken from groups B and C, while colon tissues were obtained from group A.

Tissue treatment

After tumor tissue collection, the tissues were washed three times with normal saline. A portion of the tissues was used to prepare tissue homogenate with a tissue homogenizer purchased from Shanghai Medical Instrument Factory, while the rest was subjected to extraction of primary cells for culturing. Cell growth and apoptosis were determined.

Evaluation of parameters

Tumor dimensions

When the tumors were removed, tumor diameter and volume were measured in each group (B and C), and the mean values were taken.

Protein determination

The levels of CysLTR-1 and related pathway proteins (LTD4, β-catenin and COX-2) were determined. Tumor tissues were ground in liquid chlorine, and then added to lysis buffer containing a protease inhibitor (PMSF). Next, the mixture was homogenized and spun at 500 g at 4 ^{o}C for 10 min. Then, the clear lysate was kept in a freezer at -8 ° C prior to use. Immunoblotting was applied to determine CysLTR-1 protein in tumor tissues.

Protein activity

The levels of IL-9 and IFN-γ were determined using ELISA.

Cell growth

The cultured tumor cells were made into a suspension and inoculated into a 96-well plate (100 μL/well), with 3 replicated wells for each group. Cell proliferation colorimetric assay reagent (CCK-8; 20 μL) was added to each well 2 h before the preset 12-h intervals, followed by culturing for 2 h in a 5 % carbon dioxide atmosphere. Next, absorbance (A) value at 490 nm was measured in a full-automated microplate reader, and the values were used to estimate cell proliferation.

Cell apoptosis

Apoptosis assay kit was used to detect cell apoptosis according to the kit instruction. The cells were transfected for 48 h, followed by sequential dyeing using Annexin V and PI in a 6-well plate. Then, BD FACSCalibur flow cytometer (PuDi Biotech Co Ltd Shanghai, China) was used to analyze apoptosis in the cells. The experiment was conducted in triplicate.

Statistical analysis

The SPSS 19.0 software was used for data analysis. Count data were analyzed with Chi squared test, while measurement data are expressed as mean ± standard deviation (SD), and were analyzed by *t*-test. Values of $p < 0.05$ were indicative of significant differences.

RESULTS

Basic biodata

Body weight, age, body length, indoor temperature, and indoor relative humidity were comparable amongst groups A, B and C ($p > 0.05$, Table 1).

Tumor diameter and volume

Tumor diameter and volume in group B were 21.25 ± 1.13 mm and 2.04 ± 0.43 cm^3, respectively, while the corresponding values in group C were 9.97 ± 0.81 mm and 0.67 ± 0.07 cm^3, respectively. Thus, tumor diameter and volume were markedly higher in group B than in group C ($p < 0.05$, Table 2).

Table 1: Comparison of basic profiles (mean ± SD, n = 60)

Parameter	A	B	C	F	*P*-value
Gender				0.31	0.855
Male	33	31	34		
Female	27	29	26		
Weight (g)	168.23±11.24	165.94±14.06	167.23±12.78	0.48	0.618
Age (days)	37.04±2.02	36.84±2.03	37.22±1.99	0.53	0.587
Length (cm)	17.46±1.22	17.52±1.42	17.06±1.72	1.74	0.178
Room temp(^{o} C)	23.34±1.02	23.18±0.97	23.03±1.34	1.15	0.320
Rel humidity indoor (%)	49.23±15.32	48.83±16.42	50.02±14.45	0.09	0.912

Table 2: Tumor diameter and tumor volume (mean ± SD, n = 60)

Parameter	Group B	Group C	F	*P*-value
Tumor diameter (cm)	21.25±1.13	9.97±0.81	62.84	<0.0001
Tumor volume (cm^3)	2.04±0.43	0.67±0.07	24.36	<0.0001

Levels of CysLTR-1, LTD4, β-catenin and COX-2

The levels of CysLTR-1, LTD4, β-catenin, COX-2, IL-9 and IFN-γ were markedly higher in groups B and C than in A, but were markedly up-regulated in B, relative to C ($p < 0.05$). These data are presented in Figure 1, Figure 2, Figure 3, Figure 4, Figure 5 and Figure 6.

Cell growth

Table 3 shows that from 0 to 24 h, cell growth was comparable amongst groups A, B and C. However, from 48 to 72 h, cell growth was markedly faster in B and C than in A. Cell growth in the three groups manifested significant upward trends at the different time points ($p < 0.05$).

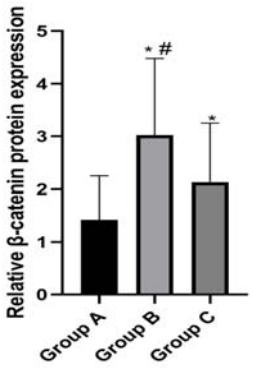

Figure 3: Comparison of β-catenin levels. β-catenin levels were markedly higher in B and C than in A, but were up-regulated in B, relative to C. *$P < 0.05$, vs A; #$p < 0.05$, vs C

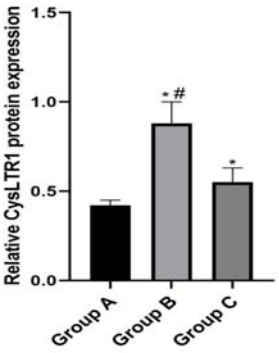

Figure 1: Comparison of CysLTR-1 levels. CysLTR-1 levels were markedly up-regulated in B and C, relative to A, but were markedly higher in B than in C

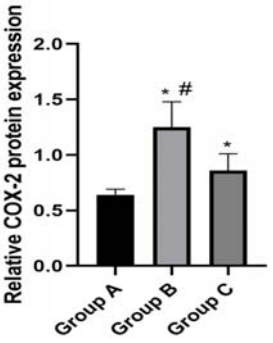

Figure 4: Comparison of COX-2 levels. COX-2 levels were markedly higher in B and C than in A, but were up-regulated in B, relative to C. *$P < 0.05$, vs A; #$p < 0.05$, vs C

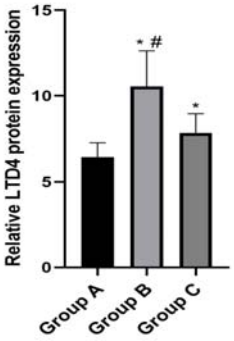

Figure 2: Comparison of LTD4 levels. LTD4 levels were markedly higher in B and C than in A, but were upregulated in B, relative to C. *$P < 0.05$, vs A; #$p < 0.05$, vs C

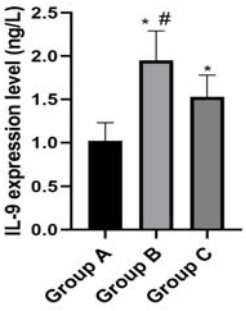

Figure 5: Comparison of IL-9 levels. IL-9 levels were markedly higher in B and C than in A, but were up-regulated in B, relative to C. *$P < 0.05$, vs A; #$p < 0.05$, vs C

Table 3: Cell growth at different time periods (mean ± SD, n = 55)

Time	Group A	Group B	Group C	*t*	*p*
24(h)	0.85±0.12	0.84±0.11	0.84±0.13	0.14	0.871
48(h)	1.04±0.23*	3.74±0.57*	1.52±0.43	663.70	<0.001
72(h)	1.65±0.44*#	5.66±0.82*#	3.68±0.67	550.30	<0.001

*P < 0.05, in comparison with value 24 h later; #p < 0.05, in comparison with value 48 h later

Table 4: Apoptotic rate (mean ± SD, n = 60)

Group	Group A	Group B	Group C	*t*	*P*-value
Apoptosis (%)	24.52±0.74	4.53±0.72	5.53±0.92	157.50	<0.001

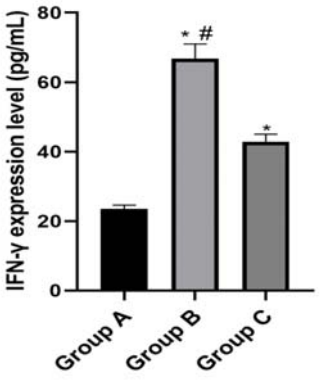

Figure 6: Comparison of IFN-γ level. IFN-γ levels were markedly higher in B and C than in A, but were up-regulated in B, relative to C. *P < 0.05, vs A; #p < 0.05, vs C

Levels of apoptosis

Apoptosis levels (%) were 24.52 ± 1.74, 4.23 ± 0.72, and 9.53 ± 0.92 in A, B and C groups, respectively. Comparison of apoptosis amongst groups A, B and C is shown in Table 4.

DISCUSSION

The determination of prognostic biomarkers for colon cancer and the treatment for specific molecular markers have been used in the management on colon cancer in recent years [10,11]. This study was aimed at investigating the effects of combination of OX and S-1 on the expression levels of CysLTR-1 and its related pathway proteins and related inflammatory cytokines (e.g. IL-9) in colon cancer. Usually, LTD4-mediated CysLTR-1 activation induces the proliferation of intestinal epithelial cells and colon cancer cells. The specific mechanism involves increase in the expressions of proteins related to cell survival (such as COX-2), and proteins involved in proliferation and migration (such as β-catenin) [12]. Therefore, levels of CysLTR-1, LTD4, COX-2, and β-catenin were determined in this study. The levels of these proteins were significantly higher in groups B and C than in control, and were significantly higher in untreated colon cancer tissue than in tissue from rats given combined treatment. In addition, the levels of the proteins were lower in the healthy colon tissues than in the colon cancer tissues. These findings suggest that these proteins play important roles in colon cancer therapy. Studies have shown that OX and S-1 have great effects on the treatment of multiple digestive system cancers such as gastric cancer, esophageal squamous cell carcinoma, and pancreatic ductal adenocarcinoma [13-15]. However, the influence of their combination on colon cancer remains unknown. Therefore, in this study, group C was treated with a combination of OX and S-1. The results showed that levels of CysLTR-1, LTD4, COX-2, and β-catenin in the treatment group were decreased after the combined treatment. From this point of view, it may, at least, be preliminarily inferred that OX and S-1 inhibited the four proteins in the rat model.

Various components of S-1inhibit gastrointestinal adverse reactions through suppression of related enzymes and blocking of phosphorylation, thereby exerting anti-cancer effects. Oxaliplatin (OX) enters cells and binds to nucleophilic molecules (including DNA, RNA, and proteins), thereby inducing apoptosis and inhibiting tumor growth [16]. Some inflammatory cytokines inhibit cell apoptosis via control of some related pathway proteins which promote cell apoptosis, and OX regulates these cytokines and reduces inflammatory responses [17]. Therefore, inflammatory cytokines, tumor size, cell proliferation, and apoptosis were studied in the rat model of colon cancer. The expression levels of IL-9 and IFN-γ were significantly higher in groups B and C than in group A, and were markedly higher in group B than the corresponding levels in group C. This shows that combined use of OX and S-1 had inhibitory effects on inflammatory cytokines in the rat colon cancer model. There was significantly longer tumor diameter in model rats than in OX-treated rats, and tumor size was significantly larger than that in group C. This indicates that the rat tumor growth was inhibited by the combined drug

treatment. There was lower cell growth in combined treatment rats than in model rats, but percentage apoptosis was markedly higher in the former. This reveals that OX, in combination with S-1 exerted an inhibitory effect on the growth of rat colon cancer cells, and increased their apoptosis. In combination with results on the levels of the above four proteins, it was found that in the rat model, after LTD4 induced increase in CysLTR-1 level, there were increases in levels of COX-2, β-catenin, IL-9, and IFN-γ, as well as % cell survival and cell proliferation; but apoptosis was decreased, resulting in relatively large tumor volume. However, combined use of OX and S-1 inhibited cell proliferation and increased tumor size in the rat model, possibly through decreases in the levels of the four proteins, reduction in percentage cell survival and cell proliferation, as well as increase in apoptosis, resulting in reduction of tumor volume and inhibition of colon cancer. These findings are consistent with those of Baba *et al* [18] who found that the combination of S-1 and oxaliplatin showed higher efficacy and stronger suppressive effect on rectal cancer, when compared with using either of the drugs only.

Study limitations

However, this research has some limitations. The effects of OX and S-1 on the rats were not separately determined. Moreover, due to limited equipment, we did not investigate the molecular mechanism underlying the effect of CysLTR-1 on the rat colon cancer model. Therefore, future studies will focus on the individual effects of OX and S-1 on the apoptosis and proliferation of colon cancer cells, and the molecular mechanism action of CysLTR-1 on colon cancer.

CONCLUSION

The combined use of OX and S-1 inhibits CysLTR-1 expression levels in rats with colon cancer. It also inhibits IL-9 expression level, thereby suppressing the proliferation and enhancing the apoptosis of the tumor cells. Therefore, the combination treatment has potentials for further development for use in clinical practice.

DECLARATIONS

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None provided.

Ethical approval

This study was approved by Chongqing Health Commission (2018MSXM010) and Science and Technology Program of Guangzhou, China.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of interest

No conflict of interest is associated with this work.

Contributions of authors

We declare that this work was done by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Yan Li and Xiongbo Guo conceived and designed the study, and drafted the manuscript. Yan Li, Bin Huang, Xiangcai Zou and Yikun Lin collected, analyzed and interpreted the experimental data. Bin Huang and Xiongbo Guo revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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REFERENCES

1. *Favoriti P, Carbone G, Greco M, Pirozzi F, Pirozzi RE, Corcione F. Worldwide burden of colorectal cancer: a review. Updates. Surg 2016; 68: 7-11.*
2. *Marley AR, Nan H. Epidemiology of colorectal cancer. Int J Mol Epidemiol Genet 2016; 7: 105.*
3. *Lukic A, Wahlund CJE, Gómez C, Brodin D, Samuelsson B, Wheelock CE, Gabrielsson S, Rådmark O. Exosomes and cells from lung cancer pleural exudates transform*

LTC4 to LTD4, promoting cell migration and survival via CysLT1. Cancer Lett 2019; 444: 1-8.

4. *Kondeti V, Al-Azzam N, Duah E, Thodeti CK, Boyce JA, Paruchuri S. Leukotriene D4 and prostaglandin E2 signals synergize and potentiate vascular inflammation in a mast cell–dependent manner through cysteinyl leukotriene receptor 1 and E-prostanoid receptor 3. J Allergy Clin Immunol 2016; 137: 289-298.*
5. *Savari S, Chandrashekar NK, Osman J, Douglas D, Bellamkonda K, Jönsson G, Juhas M, Greicius G, Pettersson S, Sjölander A. Cysteinyl leukotriene 1 receptor influences intestinal polyp incidence in a gender-specific manner in the ApcMin/+ mouse model. Carcinogenesis 2016; 37: 491-499.*
6. *Osman J, Savari S, Chandrashekar NK, Bellamkonda K, Douglas D, Sjölander A. Cysteinyl leukotriene receptor 1 facilitates tumorigenesis in a mouse model of colitis-associated colon cancer. Oncotarget 2017; 8: 34773.*
7. *Vincent JA, Wieczerzak KB, Gabriel HM, Nardelli P, Rich MM, Cope TC. A novel path to chronic proprioceptive disability with oxaliplatin: distortion of sensory encoding. Neurobiol Dis 2016; 95: 54-65.*
8. *Matsuda C, Ishiguro M, Teramukai S, Kajiwara Y, Fujii S, Kinugasa Y, Nakamoto Y, Kotake M, Sakamoto Y, Kurachi K, et al. A randomised-controlled trial of 1-year adjuvant chemotherapy with oral tegafur–uracil versus surgery alone in stage II colon cancer: SACURA trial. Eur J Cancer 2018; 96: 54-63.*
9. *World Health Organization. Principles of laboratory animal care. WHO Chron 1985; 39: 51-56*
10. *Dalerba P, Sahoo D, Paik S, Guo X, Yothers G, Song N, Wilcox-Fogel N, Forgó E, Rajendran PS, Miranda SP, et al. CDX2 as a prognostic biomarker in stage II and stage III colon cancer. N Engl J Med 2016; 374: 211-222.*
11. *O'keefe SJ. Diet, microorganisms and their metabolites, and colon cancer. Nat. Rev. Gastroenterol. Hepatol 2016; 13: 691.*
12. *Bellamkonda K, Chandrashekar NK, Osman J, Selvanesan BC, Savari S, Sjölander A. The eicosanoids leukotriene D 4 and prostaglandin E 2 promote the tumorigenicity of colon cancer-initiating cells in a xenograft mouse model. BMC Cancer 2016; 16: 425.*
13. *Bando H, Yamada Y, Tanabe S, Nishikawa K, Gotoh M, Sugimoto N, Nishina T, Amagai K, Chin K, Niwa Y, et al. Efficacy and safety of S-1 and oxaliplatin combination therapy in elderly patients with advanced gastric cancer. Gastric Cancer 2016; 19: 919-926.*
14. *Nishida N, Yamsaki M, Odagiri K, Yamashita K, Tanaka K, Sakai D, Makino T, Takahashi T, Kurokawa Y, Satoh T, et al. Combination Therapy With S-1, Oxaliplatin and Leucovorin in Patients With Advanced Esophageal Squamous Cell Carcinoma. In vivo 2019; 33: 2249-2254.*
15. *Sasaki M, Ueno H, Shiba S, Hosoi H, Okusaka T. Phase I study of S-1, irinotecan plus oxaliplatin combination therapy (S-IROX) for Unresectable pancreatic ductal adenocarcinoma. Ann Oncol 2016; 27: 36-36.*
16. *Yue B, Cai D, Liu C, Fang C, Yan D. Linc00152 functions as a competing endogenous RNA to confer oxaliplatin resistance and holds prognostic values in colon cancer. Mol Ther 2016; 24: 2064-2077.*
17. *Rui X, Pan HF, Shao SL, Xu XM. Anti-tumor and anti-angiogenic effects of Fucoidan on prostate cancer: Possible JAK-STAT3 pathway. BMC. Complement Altern Med 2017; 17: 378.*
18. *Baba H, Yamada Y, Takahari D, Matsumoto H, Yoshida K, Nakamura M, Yoshida M, Iwamoto S, Shimada K, Komatsu Y, et al. S-1 and oxaliplatin (SOX) plus bevacizumab versus mFOLFOX6 plus bevacizumab as first-line treatment for patients with metastatic colorectal cancer: updated overall survival analyses of the open-label, non-inferiority, randomised phase III: SOFT study. ESMO Open 2017; 2: e000135.*