Anticancer Activity of Cleistanthin-A Analogues on Colorectal Cancer Cells

ฤทธิ์ต้านมะเร็งของสารดัดแปลงโครงสร้างไคสแตนตินเอในเซลล์มะเร็งลำไส้ใหญ่และทวารหนัก

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ABSTRACT

Colorectal cancer (CRC) is one of the most common type of cancer worldwide including Thailand. At present, the available chemotherapeutic drug for CRC is not effective. Therefore, we investigated the anticancer effects of cleistanthin-A (cleis-A) analogues (MUC-601 and MUC-602) on human colorectal cancer cell lines. We found that MUC-601 and MUC-602 exerted a significant cytotoxic effect against CRC cells. Treatment with MUC-601 and MUC-602 significant induced SW480 cell apoptosis concomitant with the suppression of survivin expression, an anti-apoptotic protein. In addition, these cleis-A analogues markedly enhanced DNA damage. Collectively, CRC apoptosis-mediated by cleistanthin-A analogues may partly through the induction of the DNA damage, highlighting the therapeutic potential of MUC-601 and MUC-602 in CRC.

บทคัดย่อ

มะเร็งลำไส้ใหญ่และทวารหนักเป็นโรคมะเร็งที่มีอุบัติการณ์สูงทั่วโลกรวมถึงประเทศไทย ปัจจุบันการรักษามะเร็งชนิดนี้ยังไม่มีประสิทธิภาพเพียงพอ ดังนั้นการค้นคว้าหายาใหม่จึงมีความจำเป็นเร่งด่วน การศึกษาครั้งนี้มีวัตถุประสงค์เพื่อศึกษาฤทธิ์ฆ่าเซลล์มะเร็งลำไส้ใหญ่และทวารหนักของสารดัดแปลงโครงสร้างจากไคสแตนตินเอ พบว่าสาร MUC-601 และ สาร MUC-602 สามารถยับยั้งการเจริญเติบโตของเซลล์มะเร็งลำไส้ใหญ่และทวารหนักได้ดี โดยออกฤทธิ์เหนี่ยวนำให้เกิดการตายแบบอะพอพโทซิส ร่วมกับลดการแสดงออกของโปรตีน survivin ซึ่งเป็นโปรตีนที่มีหน้าที่ยับยั้งการตายแบบอะพอพโทซิส นอกจากนี้สารทดสอบยังก่อให้เกิดความเสียหายต่อสายรหัสพันธุกรรม จากผลการศึกษาจึงสรุปได้ว่าสารดัดแปลงโครงสร้างจากไคสแตนตินเอ ออกฤทธิ์เหนี่ยวนำให้เกิดความเสียหายต่อสายรหัสพันธุกรรม อันเป็นสาเหตุให้เกิดการตายแบบอะพอพโทซิส ซึ่งสารดัดแปลงโครงสร้าง MUC-601 และ สาร MUC-602 มีศักยภาพที่สามารถพัฒนาต่อไปเป็นยาเพื่อใช้ในการรักษาโรคมะเร็งลำไส้ใหญ่และทวารหนักได้

Keywords: Cleistanthin-A, Colorectal cancer, Cytotoxic effect
คำสำคัญ: ไคสแตนติน-เอ มะเร็งลำไส้ใหญ่และทวารหนัก ความเป็นพิษต่อเซลล์

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Introduction

Colorectal cancer (CRC) becomes the third leading cause of cancer-related death worldwide, and most colorectal cancer patients are detected at an advanced stage (Siegel et al., 2016). In Thailand, the incidence rate of colorectal cancer is continuously increased over the past decade and ranked as the second most common cancer after breast cancer. Generally, surgical resection remains the major treatment method for early stage of CRC whereas cytotoxic chemotherapy is extensively recommended for metastatic colorectal cancer (mCRC) patients. The most common chemotherapy regimen given for mCRC is a combination of 5-FU, leucovorin and either oxaliplatin or irinotecan with commonly used acronyms FOLFOX or FOLFIRI, respectively. Although, most of patients initially response however drug resistance is often developed later and the median survival rate of patient is only 18 to 20 months (Upadhyay et al., 2015). Thus, searching for novel cytotoxic compounds is the urgently required for treating CRC patient. Natural products are recently recognized as a source of compounds for drug discovery (Harvey et al., 2015). Of these, cleistanthin A (cleis-A), a plant-derived compound from *Phyllanthus taxodiifolius* Beille, has been extensively studied and demonstrated numerous pharmacological activities including the anticancer activity (Himakoun et al., 2011; Parasuraman, Raveendran, 2012; Wang et al., 2016). However, the detailed molecular mechanism on its anticancer activity has not been shown. In addition, the development of new anticancer agent with minimal toxicity and high efficacy that targets the molecular signaling pathway is an ultimate goal for anticancer drug development. Therefore, several rational drug designs with less cytotoxic side effect on normal cells but improvement of the absorption, distribution, metabolism, excretion and toxicity properties were reported. Indeed, previous study have reported that the structural modification of ginsenoside Rh(2) by fatty acid esterification attenuated the side effect in human normal cells (Wei et al., 2012). Therefore, in order to enhance the anticancer activity with minimal toxicity, we modified and synthesized analogues of cleistanthin-A by esterification with either butanoic acid or propanoic acid at hydroxyl group. However, the anticancer activity as well as their underlying mechanisms are not yet investigated.

DNA damage agents induce cell death by apoptosis (Roos, Kaina, 2013). DNA damage could be induced by either exogenous agents such as radiation, X-ray, UV, alkylating agents, or by the by-products from endogenous process such as reactive oxygen species (Aziz et al., 2012). To ensure cell survival, the generation of a double strand breaks (DSBs) trigger DNA damage response (DDR) pathways resulting in cell cycle arrest and activation of DNA repair mechanism. The Mre11-RAD50-Nbs1 (MRN) complex senses DNA damage and subsequent activation of the several events including phosphorylation of the histone variant H2AX, producing γ-H2AX (Podhorecka et al., 2010) which is required for the assembly of DNA repair proteins at the damaged site. The phosphorylation of the histone variant H2AX on ser139 is a key initiating step in the DDR which signals downstream to CHK1, CHK2 (checkpoint kinases) and p53 (Podhorecka et al., 2010). Later, p53 induces transcriptional activation of pro-apoptotic factors leading to apoptosis (Taylor et al., 2008). Indeed, several anticancer agents have been reported to induce DSBs-mediated the phosphorylation of histone protein H2AX such as doxorubicin, etoposide, cisplatin, and gemcitabine resulting in cancer cell apoptosis (Bonner et al., 2008). Therefore, the expression level of γ-H2AX is widely used to monitor the genotoxic effect of anticancer agents.

Survivin is a member of inhibitor of apoptosis (IAP) family that is known to negatively regulate apoptosis by inhibiting caspases cascade (Garg et al., 2016). Overexpression of survivin had been demonstrated in several types of human tumors however the expression was barely detected in normal cells. Moreover, upregulation of survivin has been frequently associated with tumorigenesis, poor prognosis, and resistance of cancer cells to chemo- or radiotherapy (Chakravarti et al., 2004; Lu et al., 2009; Yamamoto et al., 2008). In addition, survivin is suggested to play an important role during CRC progression, since the expression of survivin was increased during the normal mucosa-adenoma-carcinoma transition and maintained throughout the progression of disease (Hernandez et al., 2011). Additionally, survivin silencing potentiated anticancer effects of EGCG in human malignant neuroblastoma cells (Hossain et al., 2012). Thus, survivin is proposed to be a promising target for anticancer agent.

The present study investigated the anticancer effects of cleistanthin-A analogues (MUC-601 and MUC-602) against human colorectal cancer cell lines. Treatment with MUC-601 and MUC-602 significantly induced CRC cell apoptosis concomitant with the suppression of survivin expression. In addition, these cleis-A analogues markedly increased the expression of DNA damage marker, γ-H2AX. Collectively, cleistanthin-A analogues (MUC-601 and MUC-602) mediated CRC apoptosis partly through the induction of the DNA damage and highlighting the potential therapeutic effect of MUC-601 and MUC-602 in CRC.

Objective of the study

To investigate the anticancer activity of cleistanthin-A analogues (MUC-601 and MUC-602) as well as their underlying mechanism against human colorectal cancer cell lines.

Methodology

Cell culture, reagents and antibodies

Three human colorectal cancer cell lines (HCT-116, HT-29 and SW480) were obtained from American Type Culture Collection (ATCC) (Manassas, VA, USA) and cultured in Dulbecco's Modified Eagle's Medium high glucose (Invitrogen, Carlsbad, CA) supplemented with 10% fetal bovine serum and 1% antibiotic (100U/ml) of penicillin and 100 µg/ml of streptomycin at 37 ºC under a 5% CO_2 incubator with humidified atmosphere. The following reagents were used: Luminata™ Crescendo Western HRP Substrate (Millipore, Billerica, MA); Annexin V-FITC apoptosis detection kit BD Pharmingen (Franklin Lakes, NJ); 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and paraformaldehyde (Thermo scientific, Wilmington, UK); 4,6-diamidine-2-phenylindole (DAPI) (Roche diagnostic GmbH, Boehringer, Germany). The following antibodies were used: anti-survivin (R&D systems, Minneapolis), anti-β-actin (Sigma-Aldrich, St. Louis, MO); HRP goat anti-mouse IgG (H+L) and HRP goat anti-rabbit IgG (H+L) antibody (JIR Laboratories, Inc., West Grove, PA); anti-γH2AX antibody (Cell signaling Technology, MA), and Alexa Fluor®488 anti-rabbit IgG (H+L) (Invitrogen, Carlsbad, CA).

Cleistanthin A was purified by chromatographic techniques from a cytotoxic fraction of methanol extract of the aerial parts of *Phyllanthus taxodiifolius* Beille. The analogues of cleistanthin-A; MUC-601 and MUC-602 were modified by esterification with butanoic acid and propanoic acid, respectively at hydroxyl group. Cleistanthin-A and

its analogues were provided by Professor Dr.Patoomratana Tuchinda, Department of Chemistry, Faculty of Science, Mahidol University. The chemical structures are shown in figure 1A.

Cytotoxicity assay

Cell viability was measured by using the colorimetric MTT assay. Breifly, colorectal cancer cell lines (HCT-116, HT-29, SW480) were plated in 96-well plates for 24 h. Cells were treated with various concentrations (0.01, 0.1, 1, 5, 10, 20, 50 µM) of cleistanthin-A, MUC-601, MUC-602, ellipticine and cisplatin for 48 h and 72 h. The compounds were dissolved in DMSO and the final concentration of DMSO in the medium was less than 0.2%. After treatment, the culture medium was then carefully removed and MTT (0.5 mg/ml) solution was added and incubated at 37 ^{o}C, 5% CO_2 incubator for 4 h. Then, MTT solution was removed before adding 100 µl DMSO to dissolve the formazan crystals and measurement at an absorbance of 570 nm by Multiskan micro plate reader (Thermo Scientific, Wilmington, UK). The result was calculated as % of cell viability and IC_{50} value.

Annexin V-propidium iodide staining

SW480 cells were seeded on 60-mm petri dish for 24 h to reach 70 % confluence. Cells were then treated with 0.1, 1 and 5 µM of either MUC-601 or MUC-602 for 48 h. After incubation, cells were washed twice with cold PBS harvested and centrifuged at 1000 RPM for 5 min. The supernatants were discarded and cell pellets were stained with FITC-labeled annexin V and PI for 15 min at room temperature using Annexin V-FITC apoptosis Detection Kit according to manufacturer's instruction. A total of 30,000 events were analyzed immediately with BD FACSCantoTM flow cytometer. Annexin V and PI fluorescence signals were detected at 488/520 nm and 488/585 nm of excitation and emission, respectively. The different stages of cells were obtained from different staining patterns: viable cells (FITC-/PI-), early apoptosis (FITC+/PI-), late apoptosis (FITC+/PI+) and necrosis (FITC-/PI+) using BD FACSDiva software.

Immunofluorescence

SW480 cells were plated on cover slips in 24-well plates and incubated at 37 ºC in 5% CO_2 incubator for 24 h. After treatment with 0.1 and 1 µM of MUC-601 and MUC-602 for 48 h, the culture medium was removed and cells were washed with cold PBS containing Ca^{2+}/Mg^{2+} (PBS^{++}), fixed with 4% PFA for 20 min and permeabilized with permeabilizing buffer (0.1% triton x-100, 10% FBS and 1% BSA) for 60 min. Then, samples were incubated with anti-γH2AX antibody overnight at 4 ºC. Cells were washed with PBS^{++} for 3 times and then incubated with Alexa Fluor® 488 goat anti-rabbit IgG (H+L) antibody diluted in 10% FBS for 1 h. Cells were washed with PBS^{++} for 3 times and stained with DAPI for 10 min at room temperature and washed with PBS^{++} for 2 times. The stained cover slips were mounted and visualized at room temperature under confocal laser microscopy Olympus Fluo View FV10i (Olympus, Japan).

Western blot analysis

The expression of survivin was investigated by Western blot analysis. Briefly, SW480 cells were treated with various concentrations (0.1, 1, 5 µM) of either MUC-601 or MUC-602 for 48 h. The treated cells were washed twice with cold PBS and lysed with modified RIPA buffer containing protease and phosphatase inhibitors.

Subsequently, an equal amount of protein was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The proteins were then transferred to nitrocellulose membranes and blocked with 5 % non-fat dry milk in Tris-buffered saline with 0.1 % Tween 20 (TBST). Membranes were incubated overnight with anti-survivin and anti-β-actin primary antibodies at 4 ˚C. After washing five times with TBST, membranes were incubated with horseradish peroxidase-conjugated secondary antibody at a 1:10,000 dilution for 1 h. The signals were developed using enhanced chemiluminescence reagent with Amersham HyperfilmTM ECL.

Results

The cytotoxic effect of cleistanthin-A and its analogues on colorectal cancer cell lines

The cytotoxic effect of cleistanthin-A (cleis-A) and its analogues (MUC-601 and MUC-602) on colorectal cancer cell lines (HCT-116, HT-29, SW480) were determined by MTT assay. CRC cells were incubated with various concentrations of cleis-A and its analogues in culture medium for 48 h. As shown in Figure 1B, cleis-A and its analogues (MUC-601 and MUC-602) inhibited colorectal cancer cell growth in dose- and time-dependent manners. Among three CRC cell lines with different genetic backgrounds, the cytotoxic activity of cleis-A on HCT-116 were greater than that of it's analogues (MUC-601 and MUC-602) at 48 h. The half maximal inhibitory concentrations (IC_{50}) of cleis-A, MUC-601 and MUC-602 at 48 h on HCT-116 cells were 0.13 $\pm$ 0.07, 3.72 $\pm$ 1.09, and 1.37 $\pm$ 0.65 μM, respectively. However, the cytotoxic activity of cleis-A and its analogues (MUC-601 and MUC-602) against HT-29 is comparable. The IC_{50} values of cleis-A, MUC-601 and MUC-602 at 48 h on HT-29 cells were 0.77 $\pm$ 0.27, 0.86 $\pm$ 0.42, and 0.65 $\pm$ 0.31 μM, respectively. In addition, the cytotoxic activity toward SW480 of MUC-601 was slightly lower than that of Cleis-A and MUC-602. The IC_{50} values of Cleis-A, MUC-601 and MUC-602 at 48 h on SW480 cells were 0.22 $\pm$ 0.13, 1.49 $\pm$ 0.82, and 0.26 $\pm$ 0.07 μM, respectively. Moreover, the IC_{50} values of cleis-A, MUC-601 and MUC-602 againt three CRC cell lines at 72 h were lower than that of 48 h and showed no differences between tested compounds (data not shown). Interestingly, the IC_{50} values of ellipticine and cisplatin, the current potent anticancer compounds that used as positive control were much higher than those of cleis-A and its analogues. These results indicate that cleistanthin-A and its analogues (MUC-601 and MUC-602) have greater potential to be developed as anticancer drug for colorectal cancer treatment.

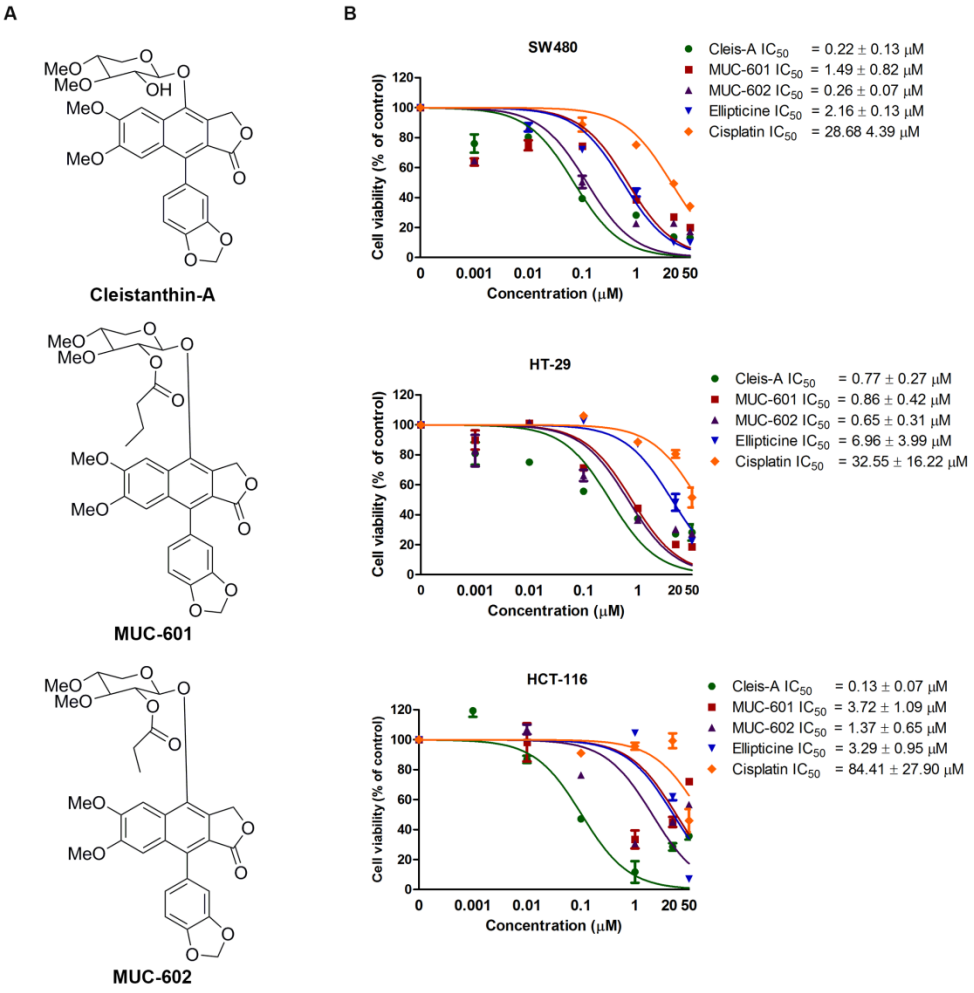

Figure 1 (A) Chemical structures of cleistanthin-A (cleis-A) and its analogues (MUC-601, MUC-602). (B) The half maximal inhibitory concentrations of cleistanthin-A and its analogues on colorectal cancer cell lines at 48 h.

Effect of cleistanthin-A analogues on apoptosis of human colorectal cancer cells

Next, we investigated the cellular mechanisms of cleistanthin-A analogues, MUC-601 and MUC-602 on CRC apoptosis by using V-FITC/PI staining and flow cytometry. As shown in Figure 2, treatment with either MUC-601 and MUC-602 for 48 h induced SW480 apoptosis in dose-dependent manner. MUC-601 at 0.1, 1, 5 μM induced late apoptosis of SW480 cells up to 23.8%, 86.3% and 88.0%, respectively. Similarly, MUC-602 at the same concentrations induced late apoptosis up to 38.1%, 88.1% and 85.9%, respectively compared to control (6.7%). The percentages of early apoptosis were only slightly increased. Likewise, ellipticine (5 μM), a known apoptosis inducer that used as positive control induced late apoptosis up to 95.7%. Thus, the result suggests that the cytotoxic activities of MUC-601 and MUC-602 on colorectal cancer cell lines (SW480) were mediated by an induction of apoptotic cell death mechanism.

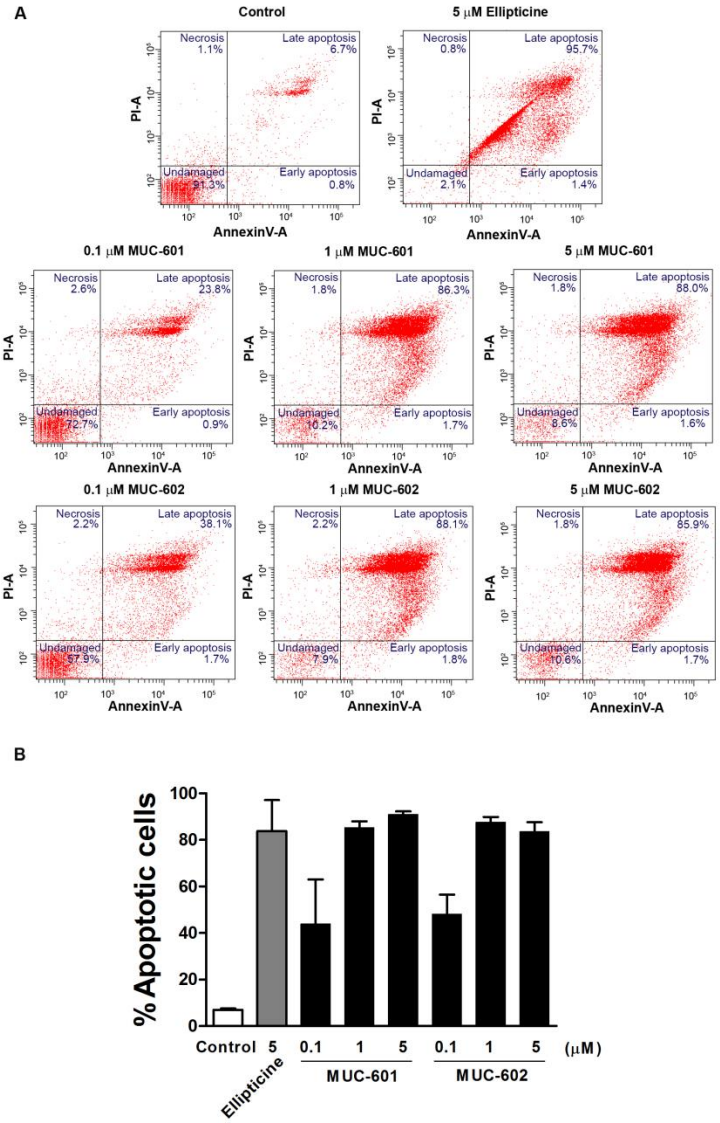

Figure 2 MUC-601 and MUC-602 induced colorectal cell apoptosis. SW480 cells were treated with various concentrations of MUC-601 and MUC-602 (0.1, 1, 5 μM) and 5 μM ellipticine for 48 h. Cells were stained with Annexin-V FITC/PI and analyzed by flow cytometry (A). The percentages of early and late apoptotic cells in each groups are shown (B). Data are means ± S.E.M. from two-independent experiments.

Cleistanthin-A analogues induce DNA damage in human colorectal cancer cells

DNA damage-induced apoptosis is reported to be the underlysing mechanism of a variety of chemotherapeutic agents. We, therefore, investigated the effects of cleistanthin-A analogues on genomic DNA integrity in CRC cells. γH2AX, a phosphorylation of histone variant H2AX at Ser^{139} is a biomarker for DNA damage especially from the double strand DNA break. In addition, the phosphorylation of H2AX is increased during early apoptosis. As shown in Figure 3, treatment with cleistanthin-A analogues, MUC-601 and MUC-602 for 48 h markedly induced DNA damage as illustrated by an increasing of H2AX phosphorylation compared to control

treatment. This result suggests that MUC-601 and MUC-602 mediated CRC apoptosis through the induction of the DNA damage.

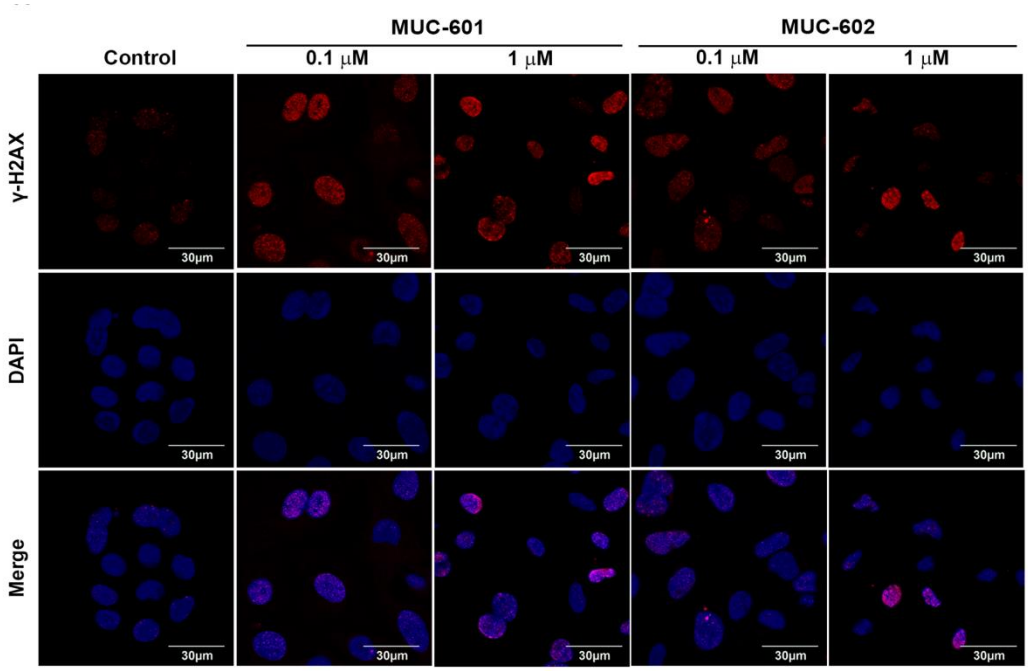

Figure 3 MUC-601 and MUC-602 induce DNA double-strand breaks in CRC cells. SW480 cells were incubated with 0.1 and 1 μM of either MUC-601 and MUC-602 for 48 h. Cells were fixed and stained with anti-γH2AX antibodies (red) and visualized under confocal microscope. The nuclei were counterstained with DAPI (blue), bar 30 μM.

Effect of cleistanthin-A analogues on the expression of apoptosis inhibitor in human colorectal cancer cells

The overexpression of survivin, the member of endogenous inhibitor of apoptosis (IAP), was implicated in apoptosis resistance to anticancer agents through its capability of inhibiting cellular caspase-3, caspase-7, and caspase-9. We next investigated whether the mechanism underlying SW480 apoptosis induced by MUC-601 and MUC-602 was involved with the regulation of survivin expression. Therefore, we investigated the effect of MUC-601 and MUC-602 on survivin protein expression in SW480 cells by western blot analysis. After treatment with either MUC-601 and MUC-602 at 0.1, 1 and 5 μM for 48 h, the expression level of survivin protein was markedly decreased in dose-dependent manner compared to the control (Figure 4). This result suggests that MUC-601 and MUC-602 induced apoptosis in SW480 through the suppression the endogenous inhibitor of apoptosis (IAP), survivin.

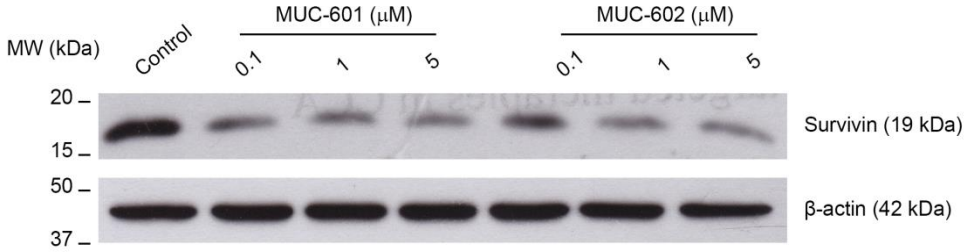

Figure 4 MUC-601 and MUC-602 suppressed the expression of survivin. SW480 cells were treated with 0.1, 1, and 5 μM of either MUC-601 and MUC-602 for 48 h. Cell lysates were then harvested with modified RIPA lysis buffer and used for western analysis using anti-survivin and anti-β-actin antibodies.

Discussion and conclusions

Colorectal cancer (CRC) becomes the third leading cause of cancer related death worldwide (Siegel et al., 2016). Despite recent advances in CRC treatment, however drug resistance is frequently developed later on resulting in tumor insensitive to the main chemotherapeutic drugs (Jensen et al., 2012). Therefore, searching for novel compounds with excellent cancer killing potential is the urgent goal of treating CRC patients. cleistanthin A (cleis-A), a plant-derived compound from *Phyllanthus taxodiifolius* Beille, has been studied and demonstrated numerous pharmacological activities, including the anticancer activity on hepatocellular carcinoma and colorectal cancer cells (Wang et al., 2016). However, the detailed molecular mechanism and specific target of this compound has not been shown. Our present study demonstrates the promising anticancer effect of two cleistanthin-A analogues (MUC-601 and MUC-602) against human CRC cell lines. Cleis-A analogues, MUC-601 and MUC-602 exerted a significant cytotoxic effect against CRC cells. Treatment with MUC-601 and MUC-602 significant induced SW480 cell apoptosis concomitant with the suppression of survivin expression, an anti-apoptotic protein. In addition, these cleis-A analogues markedly enhanced DNA damage. Collectively, cleistanthin-A analogues (MUC-601 and MUC-602) mediated CRC apoptosis may partly through the induction of the DNA damage, highlighting the potential therapeutic effect of MUC-601 and MUC-602 in CRC.

Mouting evidences suggested that apoptosis-mediated by DNA damage played a critical role in chemotherapy-induced cell death (Roos, Kaina, 2006). Double-strand DNA breaks (DSBs) were detected by ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and Rad3 related) proteins, which signals downstream to CHK1, CHK2 (checkpoint kinases) and p53 (Podhorecka et al., 2010). Later, p53 induces transcriptional activation of pro-apoptotic factors such as FAS, PUMA and BAX leading to promote the apoptosis (Taylor et al., 2008). Since DSBs can initiate the genomic instability that leads to cancer development, therefore, DSBs are closely monitored by cellular reparing system. The histone protein H2AX is a key component required for DNA repair. DSB formation induces phosphorylation of H2AX called γH2AX and is required for recruitment and assembly of DNA repair proteins at damaged site. Moreover, γH2AX also activates cell cycle checkpoint proteins to arrest cell cycle progression (Bonner et al., 2008; Podhorecka et al., 2010). Hence, the expression of γH2AX is widely used to monitor the effectiveness of cancer therapies. Indeed, several anticancer agents have been reported to induce DSBs-

mediated the phosphorylation of histone protein H2AX such as doxorubicin, etoposide, cisplatin, and gemcitabine (Bonner et al., 2008). Interestingly, treatment with cleis-A analogues, MUC-601 and MUC-602 markedly increased the phosphorylation of histone H2AX (γH2AX) suggesting the anticancer cancer activities of these tested compounds through the induction of the DNA double-strand breaks (DSBs). The formation of DSBs in cells can be caused by a variety of factors such as the direct attack on DNA by radiation, ROS, and the disruption of replication machinery mechanism (Bonner et al., 2008). Although it is not well understood how these cleis-A analogues, MUC-601 and MUC-602 induced the DNA damage in CRC cells. Previous studies have shown that cleistanthin-A inhibited vacuolar H^+-ATP ase (Wang et al., 2016; Zhang et al., 2014). Inhibition of vacuolar H^+-ATP ase-mediated the ROS production have been reported in RAW 264 and HeLa cells (Straud et al., 2010; Yokomakura et al., 2012). Therefore, the formation of DSBs in CRC cells induced by cleis-A analogues, MUC-601 and MUC-602 might be due to the inhibition of vacuolar H^+-ATP ase-mediated the ROS production.

The up regulation of survivin, the member of endogenous inhibitor of apoptosis (IAP) is observed in a variety of human cancer relative to normal tissues. Up regulation of survivin is associated with the increased of cell proliferation, apoptosis resistance and promotion of cancer angiogenesis (Garg et al., 2016). Indeed, overexpression of survivin contribution to the chemotherapeutic drug resistance has been reported in CRC cells (Lin et al., 2003). Therefore, survivin is purposed to be a promising therapeutic target for cancer treatment. Interestingly, we found that treatment with MUC-601 and MUC-602 dose-dependently reduced the expression of survivin protein in SW480 cells compared to control. It is still not known how the expression of survivin is regulated by these cleis-A analogues, MUC-601 and MUC-602. The expression of survivin can be regulated at transcriptional and post-translational levels. Recently, a plant-derived small molecule, terameprocol (EM-1421) was shown to transcriptional suppression of survivin gene expression and induced apoptosis in cancer cells (Chang et al., 2004). Further experiment is required to investigate the transcriptional activation by cleis-A analogues, MUC-601 and MUC-602. It has been shown that the expression of survivin is increased by the activation of PI3K/Akt/mTOR signaling pathway (Karar, Maity, 2011; Vaira et al., 2007). However, previous study in melanoma cells had shown that increased oxidative stress inactivated PI3K/Akt/mTOR signaling pathway (Hambright et al., 2015). Therefore, it is purposed that treatment with cleis-A analogues, MUC-601 and MUC-602 in SW480 increases ROS production that may lead to the PI3K/Akt/mTOR inactivation-mediated survivin suppression. However, further experiments are required to clarify the precise mechanistic details of MUC-601 and MUC-602 in CRC cells.

In summary, our study provides the first evidence that cleis-A analogues, MUC-601 and MUC-602 exhibit the cytotoxic effect against colorectal cancer cells at least partly mediated by the DNA damage-induced apoptosis concomitant with the suppression of survivin expression, an anti-apoptotic protein. Thus, cleis-A analogues, MUC-601 and MUC-602 has the potential to be developed as anticancer agents for cancer treatment.

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