THE ANTICANCER ACTIVITIES OF STICHOPOSIDE D ISOLATED FROM THE SEA CUCUMBER STICHOPUS CHLORONOTUS ON NTERA-2 CANCER STEM CELLS

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SUMMARY

As reported, cancer stem cells (CSCs) are in charge of dangerous characteristics including drug resistance, metastasis, recurrence and therapeutic effectiveness. Therefore, CSCs are an important target for discovering of novel effective and specific anticancer drugs. In Vietnam, the Stichopus chloronotus sea cucumber is found as a potential biological source with various active ingredients. Particularly, the active triterpene saponin stichoposide D, which was isolated from S. chloronotus, showed strong cytotoxic activity in leukemias. Herein, stichoposide D was further studied its potential anti-CSCs activities on pluripotent human embryonic carcinomas NTERA-2 cells. The compound exhibited its promising and specific cytotoxic activities in NTERA-2 cells with the IC50 = 0.26 ± 0.02 µM, in comparison with that ranging from 0.35 ± 0.02 µM to 0.53 ± 0.03 µM (P<0.05), tested on non-CSCs cancer cell lines, which were breast carcinoma (MCF-7) and lung adenocarcinoma (SK-LU-1), respectively. The working fashion of the compound on NTERA-2 cells could be apoptotic induction. Significantly, treatment of stichoposide D at 1 µM induced 76.4% of apoptotic cells as well as 1.72 relatively fold change of caspase-3 activation in comparison with the control (P<0.05). Meanwhile, stichoposide D was the first time recognized its positive efficacy on reducing the number of highly expressed CD44+/CD24+ cells, which were reported as typically CSCs characterized population. The compound also exhibited some effects on NTERA-2 cell cycle of which it arrested cells at sub-G1 phase (15.03%) and prevented those CSCs to enter the S-phase for DNA synthesis. In conclusion, stichoposide D presents potential anti-CSCs activities and should be further studied for future applications.

Keywords: apoptosis, cancer stem cells (CSCs), sea cucumber, Stichopus chloronotus, Stichoposide D

INTRODUCTION

Cancer was recognized as a serious disease worldwide, especially in the developing countries including Vietnam. The tumors persisted its unique characters by a small cell groups called as cancer stem cells (CSCs). These cells have self-renewal ability that potentially develop to heterogeneous cancer cells, substantially proliferate for expanding the malignant cell populations (Chenet et al., 2013). CSCs associate to drug resistant feature, metastasis, tumor relapse (Vinogradov and Wei, 2012; Prud’homme, 2012; Bao et al., 2013). Thus, the potential drugs targeting CSCs become more crucial and gain more attention from
scientists. Vietnam is one of the countries holding high biodiversity of marine sources that provides numerous precious natural species such as seaweed, coelenterata, sea moss, mollusks, coral, echinodermata etc. In recent years, domestic scientists have focused on Vietnamese marine species which presented various promising biological activities including anticancer cytotoxicity (Minh et al., 2012; Cuong et al., 2015; Nam et al., 2015; Ngoan et al., 2015; Ngoc et al., 2017; Thao et al., 2013; Thao et al., 2014a, Thao et al., 2014b). Stichoposide D (SC9), a component previously isolated from Vietnamese S. chloronotus sea cucumber, presents strong cytotoxic activities against various cancer cell lines (Thao et al., 2014b). Herein, the cytotoxic potents, cell cycle arresting and apoptotic inductive activities of stichoposide D targeting pluripotent human embryonic carcinomas NTERA-2 cells will be presented.

MATERIALS AND METHODS

Materials

SK-LU-1 (human lung adenocarcinoma), MCF7 (human breast carcinoma) cancer cell lines and NTERA-2 (pluripotent human embryonic carcinoma) cancer stem cell line were kind presents from Prof. JM Pezzuto, Rutgers University, USA and Dr. P Wongtrakoongate, Mahidol University, Thailand.

Cultured medium Dulbecco's Modified Eagle Medium (DMEM), fetal bovine serum (FBS), trypsin-EDTA, bovine insulin, FITC Annexin V/Dead Cell Apoptosis Kit were obtained from Invitrogen (Carlsbad, CA, USA). Caspase-3 Colorimetric Assay Kit was purchased from Biovision (Milpitas, CA, USA). Human CD44 antibody conjugated with FITC (FITC-CD44) and human CD24 antibody conjugated with PE (PE-CD24) were from Miltenyi Biotec. (Bergisch Gladbach, Germany). Other chemicals were provided by Sigma Aldrich (St. Louis, MO, USA).

Isolation of stichoposide D (SC9)

The triterpene saponin 23(S)-acetoxy-3β-hydroxy-holost-7-ene 3-O-[3-O-methyl-β-D-glucopyranosyl-(1→3)-β-D-xylopyranosyl-(1→4)-β-D-glucopyranosyl-(1→2)-[3-O-methyl-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→4)]-β-D-xylopyranoside] with the trivial name stichoposide D was obtained from the S. chloronotus sea cucumber following the previously reported chromatographic separations (Thao et al., 2014b).

Cell culture

Cells were routinely cultured in T75 flask with Dulbeco’s Modified Eagle Medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal bovine serum – FBS, 1% antibiotic (Anti-Anti solution). The MCF7 medium was further added with 0.01 mg/mL bovine insulin. The cells were sub-cultured after 3-5 days with the ratio of 1:3 and incubated at 37°C, 5% CO₂ and 100% humidified.

Cytotoxicity assay

Sulforhodamine B (SRB) cytotoxicity assay was used for screening and detecting compounds that in vitro inhibit the development of cancer cells (Skehan et al., 1990). Cancer cells and NTERA-2 cells were seeded in 96 well plate at concentration of 3×10⁴ cells/well at 37°C and 5% CO₂. After 24 hours, cells were treated with stichoposide D at different concentrations and continuously incubated for further 72 hours. Then, cells were fixed with cold trichloroacetic acid (TCA) 20% and stained with SRB (Sigma-Aldrich,) 1% (v/v) for 1 hour at room temperature (RT). The unbound SRB was washed out with 1% (v/v) acetic acid. The SRB dying protein was dissolved in 10 mM unbuffered Trisbase solution. The OD were measured at 515 nm by a microplate reader (BioTek EXL800). Ellipticine has been used as a positive control while 10% DMSO was known as negative control.
Caspase 3 induction assay

Determination of caspase 3 activities was performed using Caspase-3 Colorimetric Assay Kit of Biovision Inc. (Milpitas, CA, USA) following the manufacturer's instructions. In general, NTERA-2 cells (1×10⁶ cells/mL) after stichoposide D treatment at different concentration were lysed with 50 µL cell lysis buffer for 10 minutes to harvest total protein. Then, protein was diluted in 50 µL cell lysis buffer for each reaction. The diluted protein was added with 50 µL DTT (10 mM) and 5 µL of DEVD-pNA (200 µM) and was further incubated at 37°C for 1 hour. The OD values were obtained by microplate reader (BioTek ELX800) at 405 nm wavelength.

Cell cycle arrest and CD44/CD24 surface marker analysis using flowcytometry

NTERA-2 cells (5×10⁴ cells/mL) were cultured in 6-well plate at 37°C, 5% CO₂ in 24 hours and following by treatment with stichoposide D at different concentrations. A further incubation was lasted for 24 hours. Then, the cells were detached with 0.05% trypsin-EDTA, centrifuged at 1000 rpm in 5 minutes to obtain the cell pellets.

To determine apoptotic induction, cell pellet was resuspended with 100 µL binding buffer, 5 µL AnexinV-FITC and 1µL PI (100 µg/mL), which was incubated in 15 minutes at 37°C. An approximately 10,000 cells were undergone flowcytometry Novocyte system (ACEA Bioscience inc.) and NovoExpress sofware to eavaluate ratio of apoptotic cells.

To access cancer stem cell markers’ expression, CD44 and CD24 markers were employed. The obtained cell pellet was resuspended in DMEM containing FBS, CD44-FITC and CD24-PE antibodies at 4°C in 10 minutes, protected from light. The expression of cellular surface markers was analyzed by flowcytometry Novocyte system (ACEA Bioscience inc.) and NovoExpress sofware.

To analyze the cell cycle, the treated cells were harvested after trypsinization, washed with cold PBS 1X and fixed with 70% ethanol at 4°C in 2 hours. The fixed cells were washed with cold PBS 1X twice, incubated with RNase A (1 mg/mL) in 37°C water bath for 15 minutes and then stained with propidium iodide (1 mg/mL) in 1 hour. The cell cycle arrest was determined by analyzing 10,000 cells per each sample using flowcytometry Novocyte system (ACEA Bioscience inc.) and NovoExpress sofware.

Statistical analysis

The data was reported as mean ± standard deviation (SD), which were analyzed by the GraphPad Prism 7 software using unpaired t-test. The P< 0.05 was considered statistically significance.

RESULTS AND DISCUSSION

Isolation and chemical structured confirmation

The compound stichoposide D was re-isolated from the S. chloronotus sea cucumber following the previously reported chromatographic separations (Thao et al., 2014b). The compound was also confirmed its chemical structure (Figure 1) by comparison of its ¹³C-NMR data with those reported (Kalinovsky et al.,1984) as well as detailed analysis of its 2D NMR spectra.

Cytotoxicic activity of stichoposide D in NTERA-2 cancer stem cells

In other previous reports, the cytotoxicity of stichoposide D was examined on several cancer cell lines with IC₅₀ ranging from 1.0 to 1.5 µM (Yun et al.,2015). In this report, we experimented the growth inhibitory potent of stichoposide D in NTERA-2 cells, in comparison with other carcinomas such as MCF-7 and SK-LU-1 cells (Table 1).

Results from Table 1 showed that stichoposide D exhibited strong inhibitory activity on the growth of NTERA-2 cells with the IC₅₀ = 0.26± 0.02 µM. The compound also exhibited its ability to inhibit the proliferation of breast cancer MCF-7 cells and lung adenocarcinomas SK-LU-1 cells at much
lower IC_{50} values as 0.35± 0.02 µM and 0.53 ± 0.03 µM than those of ellipticine which were 1.42± 0.12 µM and 1.50± 0.09 µM, respectively. Stichoposide D from S. chloronotus sea cucumber demonstrated that not only inhibits cancer cells to growth but also significantly affects to cancer stem cells’ proliferation. Particularly, stichoposide D was potential for further studies on its working fashions in CSCs.

Stichoposide D triggers apoptosis in NTERA-2 cancer stem cells

The early and late apoptotic induced effects of stichoposide D on NTERA-2 cancer stem cells were determined by Annexin V – FITC and PI staining ratios, respectively. The obtained apoptotic cell numbers were showed in the Figure 2.

According to the Figure 2B, stichoposide D at 1 µM exhibited strong and significant apoptotic-induction in NTERA-2 cells, compared with that of the negative control. The compound induced up to 74.5% of FITC+/PI+ percentages cells, in comparison with 3.6% from the untreated control (P<0.05). At this concentration, stichoposide D also caused approximately 21.7% of necrotic cells, significantly compared with those were at 11.9% and 3.7% from the other two concentrations 0.5 µM and 0.25 µM (P<0.05), respectively. However, the number of apoptotic cells treated with stichoposide D at 0.25 µM and 0.5 µM was not significantly different from that of control (P>0.05).

Relating to the apoptotic pathway, caspases play essential role in the programmed cell death, in which caspase-3 catalyzes a category of major reactions. Thereby, caspase 3-inducing activity of stichoposide D on NTERA-2 cells was evaluated and presented in Figure 3.

NTERA-2 cancer stem cells, which were treated with stichoposide D at concentrations of 0.5 µM and 1 µM, were relatively enhanced 1.22 and 1.72 fold changes of caspase-3 induction comparing with the control, respectively. Park et al (2012) had reported that stichoposide D could induce apoptosis and examined the molecular mechanisms for its activity. It was found that the Figure 1. Chemical structure of stichoposide D isolated from the Vietnamese S. chloronotus sea cucumber.

Table 1. Determination of cytotoxic activities of stichoposide D on cancer cells and CSCs by SRB assay.

| Compounds       | MCF-7 (Breast carcinoma) | SK-LU-1 (Lung adenocarcinoma) | NTERA-2 (CSCs) |
|-----------------|--------------------------|-------------------------------|----------------|
| Stichoposide D | 0.35 ± 0.02              | 0.53 ± 0.03                   | 0.26 ± 0.02    |
| Ellipticine     | 1.42 ± 0.12              | 1.50 ± 0.09                   | 1.63 ± 0.12    |
compound induced apoptosis in a dose- and time-dependent manner, leading to the activation of caspases, and mitochondrial damage in experimented leukemias. Also, in accordance with another previous study which figured out the apoptotic induction by stichoposide D through activating Ceramide Synthase 6 in K562 and HL60 cells (Yun et al., 2015). Also, as reported, few promising natural compounds have been recognized to target CSCs in vivo and in vitro. These compounds play a role in sensitizing CSCs to conventional treatments, directly inducing cell death in CSCs, forcing CSCs to differentiate, or preventing CSCs from entering a dormant and more resistant state. For example, resveratrol could be able to eliminate CSC populations from tumors and to induce caspase-3/7 activated apoptosis in CD44+/CD24−/ESA+ pancreatic CSCs at 10 to 30 µM concentrations (Shankar et al., 2011). From our study, stichoposide D has confirmed its apoptotic inductive efficacy on NTERA-2 cells at very low concentration of treatment, showing the potential activity by targeting CSCs.

Effects of stichoposide D on cell cycle

The obtained result showed that stichoposide D arrested NTERA-2 cells at sub-G1 phase (Figure 4) and prevented them to enter S phase. The percentage of cells, which was arrested at sub-G1, were 15.03% for the 1 µM of stichoposide D treated group, while this number was 2.02 % for the negative control (P<0.05). In the previous report of Marzouqi et al (2011), there was frondoside A extracted from sea cucumber (Cucumaria frondosa) induced apoptosis at sub-G1. Echinoside A and D-echinoside A, which are triterpenoid glycosides isolated from Pseanothuria graeffei, caused the arrest of the cell cycle during the G0/G1 phases in HepG2 hepatocarcinomas (Zhao et al., 2012). Cucumarioside A2–2 (Cucumaria japonica) was also reported with its anticancer effects through causing cell cycle arrest during the DNA synthesis (S) in Ehrlich carcinoma mouse tumor cells (Menchinskaya et al., 2013). However, there has been no reports on the effects of stichoposide D to cancerous cell cycle, especially on NTERA-2 cancer stem cells.

Figure 2. Stichoposide D induces apoptosis in NTERA-2 cancer stem cell after 24 hours of treatment. (A) control; (B) stichoposide D at 0.25 µM; (C) stichoposide D at 0.5 µM; (D) stichoposide D at 1 µM.
Figure 3. Stichoposide D stimulates caspase-3 activity in NTERA-2 cancer stem cells after 24 hours of treatment at various concentrations. Camptothecine was treated at 2.5 µM; *$P<0.05$; **$P<0.01$.

Figure 4. Stichoposide D impacts on cell-cycle of NTERA-2 cancer stem cell. (A) control; (B) stichoposide D at 0.5 µM; (C) stichoposide D at 1 µM after 24 hours incubation; (D) Summary of percentage of cell cycles at different phases (%)

Stichoposide D affects surface markers on cancer stem cells

CD44 and CD24 alone or in combination have been reported as markers to distinguish cancer stem cells from cancer cell population (Jaggupilli and Elkord, 2012). CD44+/CD24+ expression were different among tumor cell lines but highly in breast CSCs (Ricardo et al., 2011). To further investigate the anticancer activities targeting CSCs of stichoposide D, we evaluated the expression of CD44 and CD24 by using flowcytometry analysis. The obtained results for CD44/CD24 expressed levels of NTERA-2 cells under the stichoposide D treatment by using
flowcytometrical analysis were presented in the Figure 5.

As presented in the Figure 5, expressed levels of CD44/CD24 markers on NTERA-2 cells under stichoposide D treatment were affected in some manners. The CD24+/CD44- percentage in 0.25 µM stichoposide D treated group was 51.58% compared with 44.47 % in the control group. However, this number was reduced to 33.79% in the higher concentration 0.5 µM treatment of stichoposide D. Meanwhile, the expression of CD44+/CD24+ was reduced from 2.24 % in control group to 1.37%, 1.35 % and 1.67% in the group treated with stichoposide D at concentration of 0.1, 0.25 and 0.5 µM, respectively. Besides, the compound did alter the percentage of CD44+/CD24- cell group from 46.08% to 63.69% at different concentration treatment. The other group with CD44-/CD24+ was not much affected. Among classes of natural compounds, some flavonoids and alkaloids were also reported to be promising candidates against CSCs. Beside those, the lactone antibiotic brefeldin A is another natural compound that has shown anticancer potential. Recently, brefeldin A has been shown to preferentially induce cell death and down-regulated the expression of CD44, reduced the ability of the cells to form colonies in soft agarose, and reversed the EMT. Preferential killing of putative CSCs has the potential to diminish CSC populations while limiting the side effects typically associated with chemotherapy (Tseng et al., 2014). Thus, based on obtained result, we observed some positive effects of stichoposide D on expression of CSCs’ surface markers. However, in order to conclude the working mode of the compound toward those markers, other studies should be conducted.

![Figure 5](image_url)

**Figure 5.** Effect of stichoposide D on the expression of CD44/CD24 cell surface markers at 0-0.25-0.5 μM treatment after 24 hours on NTERA-2 cells (5×10⁴ cells/mL).

CONCLUSION

Herein, stichoposide D which was re-isolated from the Vietnamese *S. chloronotus* sea cucumber, presented some promising activities against pluripotent human embryonic carcinoma NTERA-2 cells. The compound exhibited its strong cytotoxicity on CSCs with the IC₅₀ = 0.26 ± 0.024 µM, in comparison with that ranging from 0.35 - 0.53 µM on non-CSCs cell lines. Through flowcytometrical analysis as well as caspase-3 inductive assay, the compound also proved itself as a promising apoptotic inducer. Meanwhile, stichoposide D was reorgnized its
positive effects on reducing CD44+/CD24- cell numbers as well as the activity on arresting NTERA-2 cells at sub-G1 phase (15.03%).

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REFERENCES

Al Marzouqi N, Iratni R, Nemmar A, Arafat K, Al Sultan MA, Yasin J, Collin P, Mester J, Adrian TE, Attoub S (2011) Frondoside A inhibits human breast cancer cell survival, migration, invasion and the growth of breast tumor xenografts. Eur J Pharmacol 668(1-2): 25-34.

Bao B, Ahmad A, Azmi AS, Ali S, Sarkar FH (2013) Overview of cancer stem cells (CSCs) and mechanisms of their regulation: implications for cancer therapy. Curr Protoc Pharmacol Chapter 14:Unit-14.25.

Cuong NX, Vien le T, Hanh TT, Thao NP, Thao do T, Thanh NV, Nam NH, Thung do C, Kiem PV, Minh CV (2015) Cytotoxic triterpene saponins from Cercodemas anceps. Bioorg Med Chem Lett 25(16):3151-6.

Chen K, Huang YH, Chen JL (2013) Understanding and targeting cancer stem cells: therapeutic implications and challenges. Acta Pharmacol Sin 34(6):732-40.

J Prud’homme G (2012) Cancer stem cells and novel targets for antitumor strategies. Curr Pharm Des 18(19):2838-49.

Jagupilli A, Elkord E (2012) Significance of CD44 and CD24 as cancer stem cell markers: an enduring ambiguity. Clinical Devel Immunol 2012:708036.

Kalinovsky A, Maltsev I, Antonov A, Stonik V (1984) 13C NMR studies on the chemical structure of holothurian glycosides. Bioorg Khim 10(12):1655-63.

Menchinskaya ES, Pisyagin EA, Kovalchyk SN et al. (2013) Antitumor activity of cucumarioside A2–2. Chemotherapy 59: 181–191.

Minh CV, Cuong NX, Dang NH, Thao NP, Quang TH, Tung NH, Nam NH, Huing NV, Kiem PV (2012) Điểm lại các nghiên cứu hòa học và hoạt tính sinh học một số loài sinh vật biển Việt Nam trong giai đoạn 2006-2012. Vietnam Journal of Science and Technology 50(6): 825.

Nam NH, Ngoc NT, Hanh TTH, Thao NP, Van Thanh N, Cuong NX, Do TT, Huong TT, Thung DC, Van Kiem P (2015) Cytotoxic bisembranoids from the soft coral Sarcophyton pauciplicatum. Chem Pharm Bull 63(8): 636-40.

Ngoan BT, Hanh TTH, Vien LT, Diep CN, Thao NP, Thao DT, Thanh NV, Cuong NX, Nam NH, Thung DC (2015) Asterosaponins and glycosylated polyhydroxysteroids from the starfish Culex novaeguineae and their cytotoxic activities. J Asian Nat Prod Res 17(10): 1010-7.

Ngoc NT, Huong PTM, Van Thanh N, Chi NTP, Dang NH, Cuong NX, Nam NH, Thung DC, Van Kiem P, Van Minh C (2017) Cytotoxic steroids from the Vietnamese soft coral Sinularia conferta. Chemical and Pharmaceutical Bulletin:c16-00881.

Park E S, Yun S H, Shin S W, Kwak J Y, Park J Y (2012) Induction of Apoptosis and Antitumor Activity by Stichoposide D through the generation of Ceramide in Human Leukemia Cells; J. Life Sci 22(6): 760-771.

Ricardo S, Vieira AF, Gerhard R, Leitão D, Pinto R, Cameselle-Teijeiro JF, Milanезi F, Schmitt F, Paredes J (2011) Breast cancer stem cell markers CD44, CD24 and ALDH1: expression distribution within intrinsic molecular subtype. J Clin Pathol 64(11): 937-46.

Shankar S, Nall D, Tang SN, Meeker D, Passarini J, Sharma J, Srivastava RK. Resveratrol inhibits pancreatic cancer stem cell characteristics in human and KrasG12D transgenic mice by inhibiting pluripotency maintaining factors and epithelial-mesenchymal transition. PLoS One. 2011;6:e16530.

Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren JT, Bokesch H, Kenney S, Boyd MR (1990) New colorimetric cytotoxicity assay for anticancer drug screening. JNCI: J Natl Cancer Inst 82(13): 1107-12.

Thao NP, Cuong NX, Luyen BTT, Nam NH, Van Cuong P, Van Thanh N, Nhiem NX, Hanh TTH, Kim E-J, Kang H-K (2013) Steroidal constituents from the starfish Astropecten polyacanthus and their anticancer effects. Chem Pharm Bull 61(10): 1044-51.

Thao NP, Luyen BTT, Vien LT, Tai BH, Dat LD, Cuong NX, Nam NH, Van Kiem P, Van Minh C, Kim YH (2014) Triterpene saponins from the sea
cucumber Stichopus chloronotus. Nat Prod Commun 9(5):1934578X1400900505.

Tseng CN, Hong YR, Chang HW, Yu TJ, Hung TW, Hou MF, Yuan SS, Cho CL, Liu CT, Chiou CC, Huang CJ. (2014) Brefeldin A reduces anchorage-independent survival, cancer stem cell potential and migration of MDA-MB-231 human breast cancer cells. Molecules. 19: 17464–17477.

Vinogradov S, Wei X (2012) Cancer stem cells and drug resistance: the potential of nanomedicine.

Phạm Nguyễn Thị Thử nghiệm trên dòng tế bào NTExRA

HOẠT TÍNH CHỒNG BÁO GỐC UNG THƯ CỦA HOẠT CHẤT STÍCHOPOSIDE D PHẦN LẬP TỪ LOÀI HẢI SÀM STICHOPUS CHLORONOTUS THÚ NGHIỆM TRÊN ĐỘNG TẾ BÀO NTExRA-2

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TÔM TÁT

Tế bào gốc ung thư (CSCs) được xem là chịu trách nhiệm chính cho tình trạng kháng thuốc, gây di căn, tái phát và giảm hiệu quả điều trị. Vì vậy, CSCs hiện là đích quan trọng để phát triển các loại thuốc mới có tính hướng đích và hiệu quả hơn. Tại Việt Nam, loài hải sâm Stichopus chloronotus được bào chế là có nhiều hoạt chất mang hoạt tính sinh học quí. Vì dù như stichoposide D, một saponin từ loại này đã cho thấy khả năng gây độc mạnh các tế bào ung thư máu. Trong nghiên cứu này, stichoposide D đã cho thấy hoạt tính mạnh kháng tế bào CSCs ở người dòng NTExRA-2 với giá trị IC₅₀ = 0.26 ± 0.02 µM, khi giá trị này là 0.35±0.02 µM trên tế bào ung thư vú (MCF-7) và 0.53±0.03 µM ở tế bào ung thư phổi (SK-LU-1). Tác dụng này có thể do khả năng gây ra sự chết tế bào theo chương trình (apoptosis). Ở nồng độ 1 µM, stichoposide D đã cản ức được 76,4% tế bào apoptosis, là tương 1,72 lần mức hoạt động của enzyme caspase-3 so với đối chứng (P<0.05). Stichoposide D cũng làm lâu được ghi nhận tác động tới số lượng tế bào biểu hiện CD44⁺/CD24⁺, những dấu ấn bê mặt đặc trưng cho nhiều loại CSCs. Hoạt chất cũng cho thấy khả năng bất tử tế bào NTExRA-2 ở pha sub-G1 đạt 15,03%, ngăn chặn chúng bước vào pha S là pha tổng hợp ADN. Như vậy, stichoposide D đã cho thấy những hoạt tính tiềm năng kháng CSCs và cần được nghiên cứu kĩ hơn về cơ chế tác động phân tử để có thể ứng dụng trong tương lai.

Từ khóa: tế bào gốc ung thư, hải sâm, Stichopus chloronotus, stichoposide D, apoptosis

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Yun S-H, Park E-S, Shin S-W, Ju M-H, Han J-Y, Jeong J-S, Kim S-H, Stonik VA, Kwak J-Y, Park J-I (2015) By activating Fas/ceramide synthase 6/p38 kinase in lipid rafts, stichoposide D inhibits growth of leukemia xenografts. Oncotarget 6(29): 27596.

Zhao Q, Xue Y, Wang J et al (2012) In vitro and in vivo anti-tumour activities of echinoside A and ds-echinoside A from Pearnonothuria graeffei. J Sci Food Agric 92: 965–974