Original Article

The Effect of Sea Cucumber Extract (*Holothuria scabra*) on the Proliferation of Human Placenta Derived Mesenchymal Stromal Cells

Kornthong N, PhD[1,4], Saengsuwan J, MSc[1], Duangprom S, MSc[1], Songkoomkrong S, MSc[1], Vivattanasarn T, BSc[1], Suwansa-ard S, PhD[2], Manochantr S, PhD[3,4], Sobhon P, PhD[5]

[1] Chulabhorn International College of Medicine, Thammasat University, Pathumthani, Thailand
[2] Faculty of Science, Health, Education and Engineering, University of the Sunshine Coast, Maroochydore, Queensland, Australia
[3] Division of Cell Biology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Pathumthani, Thailand
[4] Center of Excellence in Stem Cell Research, Thammasat University, Pathumthani, Thailand
[5] Department of Anatomy, Faculty of Science, Mahidol University, Bangkok, Thailand

Background: The sea cucumber, *Holothuria scabra,* is an economically important aquatic species. They have received considerable attention because of their self-regeneration ability. They may contain numerous growth factors necessary to drive the proliferation and differentiation of stem cells for tissue maintenance. The knowledge of regenerative process, including the factors that regulate this process, may provide a new treatment option for degenerative diseases in human.

Objective: The present study focused on the effects of sea cucumber extract (*H. scabra*) on the proliferation of mesenchymal stromal cells (MSCs) derived from human placenta.

Materials and Methods: The *H. scabra* crude protein extracts were prepared from the body wall (BW) and viscera (VI) using 0.1 M phosphate buffer saline (PBS) and 0.1M acetic acid buffers. MSCs were isolated from the human placenta using enzyme digestion. The effects of *H. scabra* extracts on cytotoxicity and MSC proliferation were evaluated using MTT assay and cell counting, respectively.

Results: The SDS-PAGE showed abundance of proteins in the BW and VI extracts using 0.1M PBS buffer. Less abundant proteins were observed in the tissues extract using 0.1M acetic acid buffer. However, proteins with molecular weight of ~38 kDa and ~17 kDa were highly detected in BW. The *H. scabra* protein extracts at low doses did not show any toxicity to PL-MSCs, moreover they could increase the cell number at the range of 0.01 µg/ml to 25 µg/ml. The treatment of 0.1 and 1 µg/ml of *H. scabra* extracts increased the proliferative rate of MSCs when compared with control.

Conclusion: These results obtained an *in vitro* proliferative potency of the *H. scabra* extracts on MSCs derived from human placenta. While further studies are required, this finding has provided the evidence that the sea cucumber extracts could be potentially used to induce *in vitro* MSC proliferation.

Keywords: *Holothuria scabra*, Mesenchymal stromal cells, Sea cucumber, Proliferation

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The sea cucumber, *Holothuria scabra*, is one of economically important aquatic species, which is found in coastal areas and naturally distributed in Asian countries, including China, Japan, Malaysia, Thailand, Vietnam, Indonesia and Philippines[1]. Sea cucumber has been used as traditional medication and nutritional supplement, especially in the Asian countries[2,3]. Thus, the market demands of sea cucumbers are rising due to strong consumers' favor for this sea food. To date, several studies have supported the medicinal properties of sea cucumber extracts, including wound healing, antimicrobial, antifungal, anticancer, and immunomodulation[4-7]. Based on taxonomy, sea cucumbers belong to phylum Echinodermata, which also includes the sea urchins and starfish. Like starfish, the sea cucumbers have an ability to regenerate their body parts following self-induced or traumatic mutilations, and for asexual reproduction[8]. The regenerative process of this animal indicated a great capacity of de-differentiation and re-differentiation, which are more limited in humans and most of mammals[9,10]. The regenerative process has been well documented in various species of echinoderms, including the regeneration of visceral organs after evisceration in the sea cucumbers[11]. However, the molecular pathway underlying this process is not fully understood. There are several reports on the sea cucumber transcriptomic databases. It has been

Correspondence to:
Manochantr S.
Division of Cell Biology, Department of Preclinical Sciences, Faculty of Medicine, Thammasat University, Pathumthani 12121, Thailand.
Phone: +66-2-9269710, **Fax:** +66-2-9269755
E-mail: bsirikul@gmail.com

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suggested that the intercellular signaling pathways and many important genes have been expressed during regeneration of *Apostichopus japonicas* and *Holothuroidea glaberrima*[12-14]. Therefore, the molecular pathways that are involved in sea cucumber regeneration may expand the research fields of therapeutic possibilities on human degenerative disorder.

Mesenchymal stromal cells (MSCs) are adult stem cells and can be isolated from various sources including bone marrow, adipose tissue, umbilical cord, chorion and placenta[15-18]. MSCs contain the unique ability to self-renew and multipotential differentiation capacity into various mesodermal cell lineages, including osteoblast, chondrocyte, myocyte and adipocyte, as well as non-mesodermal tissues, such as cardiac muscle, neuron and skin[19-21]. Furthermore, MSCs have been proposed as tool for new therapeutic strategy in degenerative disorders such as osteoporosis and Alzheimer's disease, with an increasing number of studies demonstrating ability of both bone marrow derived MSCs and postnatal tissue-MSCs[22].

A number of previous works have shown the functional activity of sea cucumber extract on various cell types. The sulphated polysaccharides extracted from the BW of *Stichopus japonicus* could not only increase cell viability and proliferation of neural stem/progenitor cells[23], but also enhance a neurosphere formation[23]. The BW of sea cucumber contains a fucan sulfate which has been shown to be a potent inhibitor of osteoclastogenesis[24]. It is also a source of saponin compound[25] and one form of this compound, the philinopside E, has been proved for its anti-angiogenesis and anti-tumor activity both *in vitro* and *in vivo*[26]. Moreover, 106 chemicals together with their 26 potential targets, which potentially have pharmacological effects on health improvement and disease treatments, have been identified in the sea cucumber extracts[27]. Collectively, those identified bioactive molecules are mostly the derivatives of triterpenoid and steroid glycosides, which belong to the carbohydrate and fatty acid classes. However, the amino acid-based molecules, which are derived from the sea cucumbers and potentially have pharmacological effects, is still understudied. In the present study, we hence investigated the protein profile of the sea cucumber extracts as well as studied the effect of these protein extracts on the proliferation of MSCs. We hypothesized that the sea cucumber proteins, which is rich in various types of growth and differentiating factors, may act as exogenous agents to stimulate or help to improve MSCs proliferation activity and thus may be applicable in some degenerative diseases of human.

Materials and Methods

Animal and protein extraction

The sea cucumbers (*Holothuria scabra*) with 200 to 500 g body weight were obtained from the Prachuapkirikhan Coastal Fisheries Research and Development Center, Thailand. The experiments were performed according to the guidelines on the care and use of animals for scientific purpose provided by the Institutional Care and Use Committee of Thammasat University. This study was specifically approved by Animal Care and Use Committee of Thammasat University, National Research Council of Thailand (NRCT), Protocol Number 019/2561. All efforts were made to minimize the suffering of animals. The animals were anesthetized by being immersed in the clove oil water (200 µL/L of clove-oil in seawater) for 10 to 15 min. The body wall (BW) and viscera (VI) were then collected and cut into small pieces. For crude protein extraction, each tissue was homogenized in the different lysis buffers, either 0.1 M phosphate buffer saline (PBS; 3.35 M NaCl, 0.08 M NaH_2PO_4, 0.03 M KH_2PO_4 in distilled water) or 0.1 M acetic acid, which were added with a protease cocktail inhibitor (AMRESCO, USA). The homogenates were subsequently centrifuged at 12,000xg at 4°C for 30 min. The supernatants were carefully collected and stored at -80°C until use.

Protein profile and protein measurement

The protein extracts from different tissues were measured for their concentrations by Bradford assay in which the bovine serum albumin (BSA) was used as a standard control. The protein profiles of BW and VI extracts were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Briefly, 15 µg of crude proteins were loaded and separated through 15% polyacrylamide gel (Bio-Rad, USA) using gel electrophoresis. The electrophoresed proteins were visualized by staining with Coomassie Brilliant Blue R-250 following a routine protocol described previously[28].

Mesenchymal stromal cells isolation and culture

Postpartum placentas, which have been screen for negative from any infectious diseases, were collected from normal vaginal delivery or cesarean section at Thammasat Chalermprakiat Hospital. This study was approved by the Human Research Ethics Committee of Thammasat university No. 1 (Faculty of Medicine: Number of COA 043/2019). All subjects participated in the study after written informed consents. Samples were kept in 0.1 M PBS solution at room temperature during transportation. Placenta was cut into small pieces and digested in medium containing 1.6 mg/ml collagenase XI and 200 mg/ml deoxyribonuclease I (Sigma-Aldrich, USA) at 37°C for 3 h. The isolated cells were washed twice with 0.1 M PBS and cultured in completed medium containing Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine and 100 U/ml penicillin and 100 µg/ml streptomycin (Gibco, USA). The cultures were maintained in humidified incubator set to 5% CO_2 at 37°C. The culture medium was replaced every 3 to 4 days. For sub-culturing, the unattached cells were removed whereas the adherent cells were collected by the treatment with a 0.25% trypsin EDTA solution (Gibco, USA) for 3 min before centrifugation at 500 g for 5 min. The placenta-derived MSCs (PL-MSCs) were resuspended in completed medium before seeding in triplicate in new flasks with a density of $1x10^4$ cells/cm^2.

Characterization of placenta derived mesenchymal

stromal cells isolation

After cultured for 3 to 4 passages, the cells were characterized for their immunophenotype and differentiation potentials. For osteogenic induction, $4.5x10^4$ isolated PL-MSCs were seeded into 24-well plate and incubated at 37°C, 5% CO_2 for overnight. Osteogenic differentiation was induced by addition of DMEM containing 10% FBS plus100nM dexamethasone, 10 mM β-glycerolphosphate (Sigma, USA) and 50 µM ascorbic acid 2-phosphate (Sigma, USA) for 21 to 35 days. The medium was replaced every 2 to 3 days. The level of osteogenic differentiation was assessed by alizarin red staining (Sigma, USA). The differentiated MSCs were observed under the microscope (Nikon TS100, Japan.). MSCs cultured in complete DMEM without osteogenic differentiation stimuli was served as controls.

For adipogenic induction, $3x10^4$ cultured MSCs were cultured in 24-well plate and incubated at 37°C, 5% CO_2 for overnight. Consequently, adipogenic differentiation was triggered using DMEM plus 0.2 mM indomethacin, 25 mM glucose, 1 mM dexamethasone, and 1 µg/ml insulin. The medium was changed every 3 days until 3 to 4 weeks. The cells were stained with 0.5% oil red o (Sigma-Aldrich) and observed under the microscope (Nikon TS100, Japan). Control was cultured in complete DMEM without any adipogenic stimuli. To examine the immunophenotype of cultured PL-MSCs, PL-MSCs at passage 2 to 5 were incubated with 10 µl of fluorescein isothiocyanate (FITC)- or phycoerythrin (PE)-conjugated antibodies against CD34 (BD Bioscience, USA), CD45 (BD Bioscience, USA), CD73 (BD Bioscience, USA), CD90 (AbD Serotec, USA) or CD105 (BD Bioscience, USA). The positive cells were identified by comparison with isotype-match controls [FITC-conjugated mouse immunoglobulin G1 (IgG1) and PE-conjugated mouse immunoglobulin G2a (IgG2a)]. At least twenty thousand label cells were acquired and analyze using flow cytometry (FACScalibur™, Becton Dickinson, USA) and CellQuest® software (Becton Dickinson, USA).

Treatment of sea cucumber extracts, cytotoxicity test, and cell proliferation of PL-MSCs

PL-MSCs at the 3rd passage were plated in 96-well plate (Costar, USA) with the density of $3x10^3$ cells/cm^2 and allowed to attach for 24 h in the completed medium. To synchronize the cell stage, the completed medium was removed and the serum free medium was added into the culture plate for 24 h. The different concentration (0.01, 0.1, 0.5, 1, 5, 10, 25, 50, and 100 µg/ml) of BW and VI extracts were added into the completed medium. At the 24 h, 72 h, and 120 h after treatment, PL-MSC proliferative rate and cytotoxicity after completion of various treatment times were measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Briefly, the MTT (Sigma-Aldrich, Saint Louis, MO, USA) stock solution (5 mg/ml in PBS) was added directly into the MSC plate culture to the final concentration of 0.5 mg/ml. Subsequently, MSCs plate culture was incubated in the dark at 37°C for 4 h and then MTT solution was discarded from the MSC plate culture. To dissolve the formazan crystal, 100 µl solubilizer (0.1% Triton x-100, 0.1 N HCl in isopropanol) was added into the MSC plate culture. The plate culture was measured for optical density (OD) by the automatic microplate reader at 570 nm and 630 nm.

Growth kinetic of PL-MSCs

The PL-MSCs in passage 3 was seeded at a density of $1x10^3$ cells/cm^2 in 24-well plates (Costar, Corning, USA). The experiments were divided into 3 groups: (1) PL-MSCs cultured in completed medium which served as a control; (2) and (3) PLMSCs treated with BW and VI extracts, respectively, at different concentrations (0.1, 1, 10, 25 µg/ml) for 10 days. The experiments were performed in triplicate. For cell harvesting, the cells were disaggregated by 0.25% trypsin EDTA solution and counted every 2 days until 10 days. Cell growth of sea cucumber-treated PL-MSCs was then compared to that of the control which was the PL-MSCs cultured in completed medium without *H. scabra* extracts. The number of cells were recorded and the statistical difference between each group was tested.

Statistical tests

Statistical significance analyses were performed with a SPSS program (Statistical Product and Service Solutions; version 11), using a one-way analysis of variance (ANOVA). A probability value less than 0.05 ($p<0.05$) indicated a significant difference.

Results

Protein profile of H. scabra extracts

In this study, four different *H. scabra* extracts were studied including the BW extracted by 0.1M PBS (BW-PBS) and 0.1 M acetic acid (BW-AA), VI extracted by 0.1 M PBS (VI-PBS) and 0.1M acetic acid (VI-AA). The results of the SDS-PAGE analysis of total protein extracted from *H. scabra* BW and VI using different types of lysis buffers were shown (Figure 1). The most striking difference when using different lysis buffers was the protein yield obtained from the same tissue. However, the use of different lysis buffers for the same tissue resulted in a similar protein profile outcome. Prominent protein bands were observed for both BW-PBS and VI-PBS extracts. BW-PBS extract showed five bands with molecular weight of >170, ~120, ~45, ~39 and ~17 kDa , whereas BW-AA extract showed two bands with molecular weight of ~39 and ~17 kDa. In the VI-PBS extract, the proteins with molecular weight of ~17 kDa and ~11 kDa were detected, although a faint smear of protein was observed. However, no band was observed for the VI-AA extract, except for a weak protein band with molecular weight of ~11 kDa (Figure 1). Comparing between two different tissues, the protein extracts derived from BW and VI displayed a different protein profile which are not surprised as these tissues have a distinct physical structures and physiological functions. However, the protein components within these two tissues should be investigated further, especially ones with high abundance.

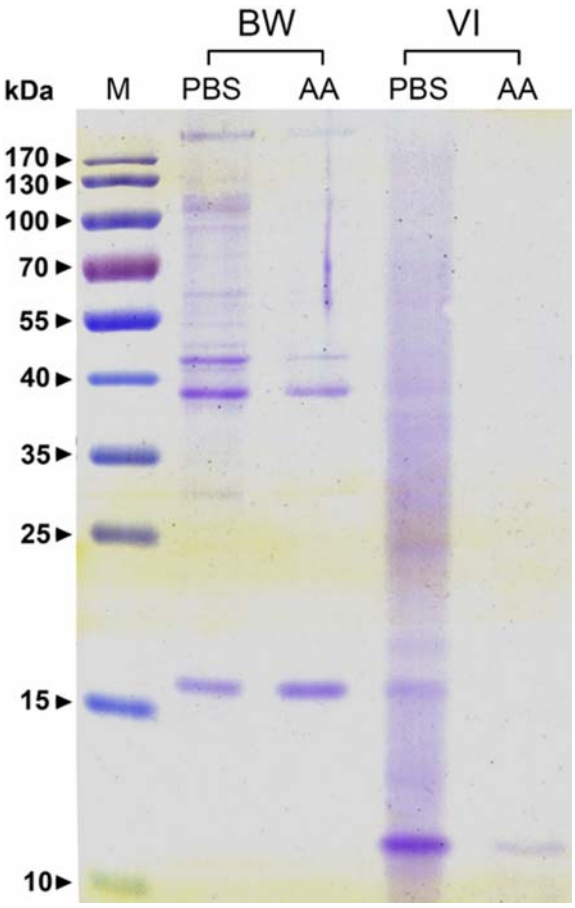

Figure 1. The SDS-PAGE showed the bands of protein isolated from body wall (BW) and viscera (VI) using 0.1 M PBS comparing with 0.1 M acetic acid. Lane 1: Body wall extracted with 0.1M PBS (BW-PBS), Lane2: Body wall extracted with 0.1 M acetic acid (BW-AA), Lane3: Viscera extracted with 0.1 M PBS (VI-PBS), Lane 4: Viscera extracted with 0.1 M acetic acid (VI-AA).

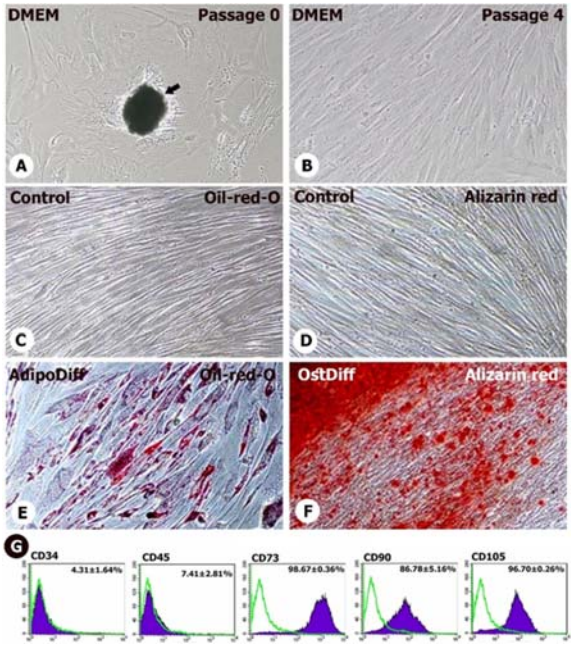

Figure 2. The characteristic of placenta-derived mesenchymal stromal cells (PL-MSCs). (A) The PL-MSCs isolated from the placenta (arrow) display a fibroblast-like morphology at passage 0. (B) Homogenous spindle-shaped MSCs at passage 4. (C-F) PL-MSCs could differentiated into adipocytes and osteoblasts as evidence by the red color after oil-red-o and alizarin red staining, respectively. PL-MSCs cultured in completed medium served as controls. (G) Flow cytometry demonstrated the expression of MSC markers including CD73, CD90, CD105 and hematopoietic markers including CD34 and CD45.

Characteristic of placenta derived mesenchymal stromal cells

MSCs isolated from human placenta exhibited fibroblast-like cells after cultured for 3 to 5 days (Figure 2A). After sub-culture for 3 to 4 passages, the spindle-shaped cells were homogenous and had a high proliferative capacity (Figure 2B). After induction into adipocyte,the spindle-shaped MSCs were changed to bulky cell with many lipid droplets in cytoplasm. These cells showed positive signals when staining with oil-red-o (Figure 2E). For osteogenic induction, cells exhibited positive signal of alizarin red staing after induction for 28 days (Figure 2F). On the other hand, there is no positive signals of oil-red-o (Figure 2C) or alizarin red (Figure 2D) in controls. For their immunophenotype, these cells expressed MSC markers including CD73, CD90, CD105 and did not express hematopoietic markers including CD34, CD45 (Figure 2G).

Cytotoxicity determined by MTT assay

To test whether *H. scabra* protein extracts have any cytotoxic effect, the viability of PL-MSCs cultured with the extracts was evaluated by MTT assay. The treatment of BW-PBS extract at the concentrations of 0.1 to 50 µg/ml could enhance MSC proliferation (Figure 3A). Their stimulatory effects were early observed at day 1 when using 0.1 to 50 µg/ml extracts. In contrast, the use of BW-PBS at concentration of 100 µg/ml decreased the percentage of cell proliferation. For BW-AA treatment, the proliferative effect could be observed when the PL-MSCs were treated with this extract at concentrations of 0.01 to 50 µg/ml, however, the

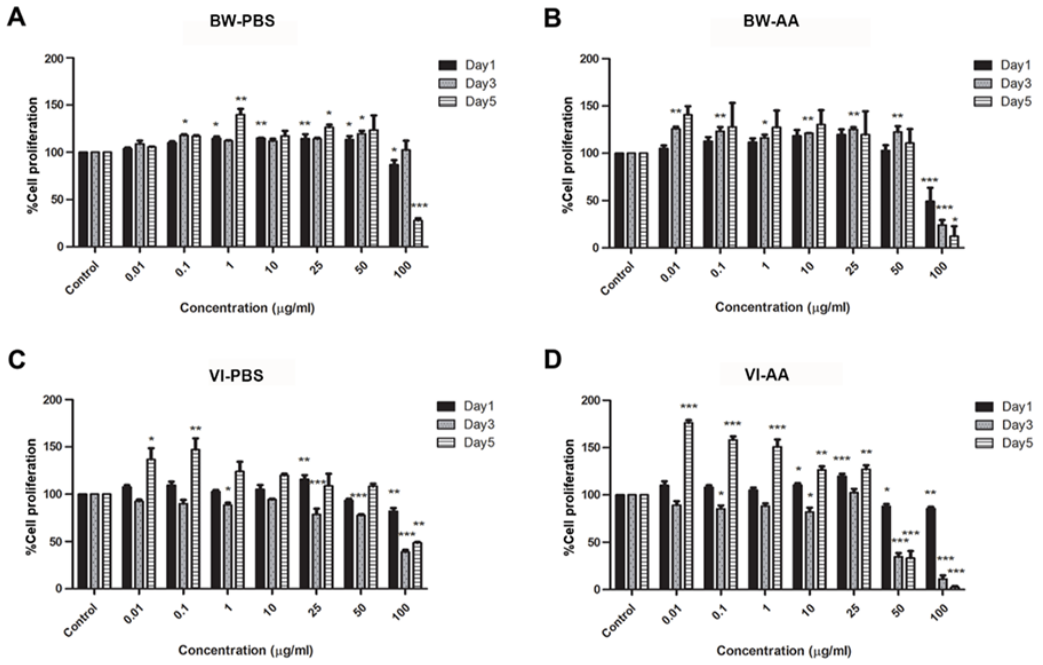

Figure 3. MTT assay of PL-MSCs after treated with the sea cucumber (*H. scabra*) extracts at days 1, 3, 5. (A) PL-MSCs treated with the body wall (BW) extracted using 0.1 M PBS. (B) PL-MSCs treated with the body wall (BW) extracted using 0.1 M acetic acid. (C) PL-MSCs treated with the viscera (VI) extracted using 0.1 M PBS. (D) PL-MSCs treated with the viscera (VI) extracted using 0.1 M acetic acid. The Y-axis show the percentage of cell growth whereas the X-axis show the concentration of sea cucumber extracts used for PL-MSC treatment. Each bar graph represents the mean ± SEM of 3 independent experiments (n = 3). Statistical differences are indicated by asterisks (* p<0.05, ** p<0.01, *** p<0.001).

MSC proliferation was declined when the concentration of extract was increased (Figure 3B). Similar to BW-PBS, the treatment with 100 µg/ml BW-AA resulted in a decrease of MSC proliferation. Both BW-PBS and BW-AA could increase MSC proliferation during the treated period. The most effective condition when using BW-PBS is the use of 1 µg/ml for 5 days (approximately 0.5-fold increase when compared with the control). For BW-AA, the proliferative effect was highest when the PL-MSCs were treated with 0.01 µg/ml for 5 days. Collectively, we suggested that the use of very low concentration of BW extracts, as low as 0.01 µg/ml, was most effective to enhance *in vitro* PL-MSCs proliferation. On the other hand, the use of very high concentration decreased the percentage of cell proliferation. This suggested that the high concentration (100 µg/ml) was toxic to the PL-MSCs and possibly induced cell death.

Although the protein profile of VI extracts were not prominent, their stimulatory effect on PL-MSC proliferation was promising, especially when the cells were treated with low concentrations (0.01 to 0.1 µg/ml) for 5 days (Figure 3C and 5D). However, MSC proliferation was decreased following increased concentrations varying from 1 to 100 µg/ml. We also found that the VI extracts could induce MSC proliferation when the duration of treatment was prolonged until day 5. This suggested that the exposure time might be a critical factor for the VI extracts. The use of acetic acid buffer for VI extraction showed a better result of MSC proliferation, suggesting that the potent molecules were obtained by this lysis buffer rather than the 0.1 M PBS. In comparison between different tissues, we found that the VI extracts were the most potent candidates for inducing PL-MSC proliferation, especially the use of 0.01 µg/ml VI-AA for 5 days.

Cell growth determined by direct cell counting

Apart from MTT assay, we also investigated the effect of sea cucumber extracts on the growth kinetic of PL-MSCs. Low doses, ranging from 0.1 to 25 µg/ml, were used within this experiment. The cell number of PL-MSCs in each group was slowly increased from day 0 to day 6 and increased rapidly from day 8 to day 10 (Figure 4A to D). However, we found that almost all extracts had no effect on PL-MSC growth during the cultured period, excepted for BW-PBS treatment at day 10 (Figure 4A to D). For the treatment of PL-MSCs with BW-PBS, the result at day 10 indicated that the use of BW-PBS at concentration of 1 µg/ml could significantly increase MSC growth when compared with the control (Figure 4A). However, the decreased cell

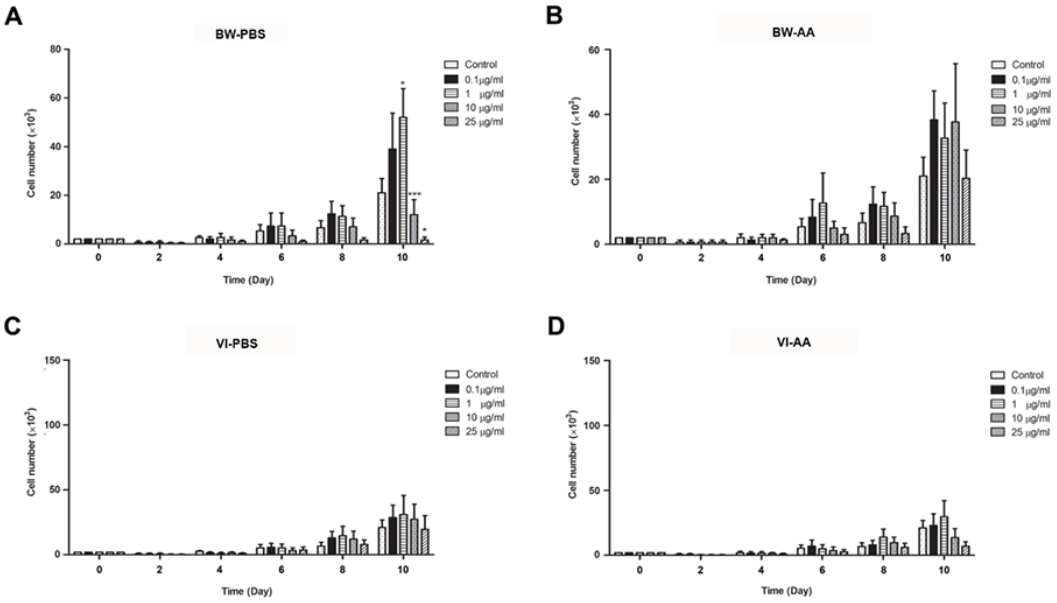

Figure 4. Growth kinetics of PL-MSCs treated with the sea cucumber (*H. scabra*) extracts for 10 days. (A) PL-MSCs treated with the body wall (BW) extracted using 0.1 M PBS. (B) PL-MSCs treated with the body wall (BW) extracted using 0.1 M acetic acid. (C) PL-MSCs treated with the viscera (VI) extracted using 0.1 M PBS. (D) PL-MSCs treated with the viscera (VI) extracted using 0.1 M acetic acid. The Y-axis show the percentage of cell growth while the X-axis show the concentration of sea cucumber extracts used for PL-MSC treatment. Each bar graph represents the mean ± SEM of 3 independent experiments. * $p<0.05$, ** $p<0.01$, *** $p<0.001$). Statistical differences are indicated by asterisks.

growth was observed when using 10 and 25 µg/ml of BW-PBS extracts. In addition, the cell numbers of PL-MSCs also increased when using 0.1, 1 and 10 µg/ml of BW-AA, however, there was not statistically significant difference compared to control (Figure 4B). For the treatment of PL-MSCs with VI-PBS and VI-AA, it showed that the cell numbers of PL-MSCs was not different from the control group (Figure 4C to D).

Discussion

From this study, we found that the sea cucumber protein extracts from the BW and VI could enhance the PL-MSC proliferation. Therefore, the potent protein molecules within these extracts were expected. The investigation of nutritional quality and composition of sea cucumber demonstrated a high proportion of total and essential amino acids[29,30]. It has been shown that the amino acids such as glycine, glutamic acid, and arginine, could induce lymphocyte proliferation and activity[31]. Therefore, these amino acids present in the *H. scabra* extracts may be important candidates that are involved in the proliferation of PL-MSCs. Generally, highly insoluble collagen fibers constitute approximately 70% of the total body wall protein[12,32]. Interestingly, the pepsin-solubilized collagen extract of *Stichopus japonicus* could enhance human keratinocyte cell migration and proliferation[33]. However, it is likely that the *H. scabra* extracts used in the current study, particularly the BW, contains less amount of collagen because the tissues were not treated with alkaline solution and hydrolysis enzymes during extraction, which are important steps for collagen extraction[32,33]. Growth factors, including fibroblast growth factor (FGF), epidermal growth factor (EGF), bone morphogenetic protein (BMP), transforming growth factor beta (TGFβ), have been known to mediate proliferation in multipotential stromal cells[34,35]. These cytokine proteins are also present in the sea cucumbers and they play important role in the regeneration and development of this animal[12-14]. Potentially, the sea cucumber cytokines may act as exogenous agents to stimulate the proliferation of PL-MSCs in the current study. Whether the particular molecules are presented in the *H. scabra* extracts and its association with PL-MSC proliferation are challenging and require further investigation.

Conclusion

In this study, we have revealed the protein profiles of the *H. scabra* extracts which were obtained from different extraction methods. The extracts showed a proliferative effect on the human PL-MSCs. This provided a foundation for further investigations of the putative proteins within the sea cucumber extract that can be used as an alternative pharmaceutical agent for the prevention and treatment of

age-related degenerative disorder.

What is already known on this topic?

Sea cucumbers have been used as traditional medication and nutritional supplement for long time. Several studies have supported the medicinal properties of sea cucumber extracts, including wound healing, antimicrobial, antifungal, anticancer, and immunomodulation. Sea cucumbers have the special capacity to regenerate their tissue after injury. The regenerative process of this animal indicated a great capacity of de-differentiation and re-differentiation, which are limited in humans.

What this study adds?

The *H. Scabra* extracts at concentration of 0.1 to 1 µg/ml could enhance the proliferation of human placenta derived mesenchymal stem cells.

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Potential conflicts of interest

The authors declare no conflicts of interest.

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สารสกัดโปรตีนปลิงทะเลขาว (*Holothuria scabra*) ต่อกระบวนการแบ่งเซลล์ต้นกำเนิดมีเซนไคม์ที่ได้จากรก

นภมณี คอนทอง, จุฑารัตน์ แสงสุวรรณ, สุภาวดี ด้วงพรม, สินีนาฎ ทรงคุ้มครอง, ธิปก วิวัฒน์ธนสาร, เสาวรส สุวรรณสะอาด, ศิริกุล มะโนจันทร์, ประเสริฐ โสภณ

ภูมิหลัง: ปลิงทะเลขาว (*Holothuria scabra*) นับว่าเป็นสัตว์น้ำเศรษฐกิจที่สำคัญและได้รับความสนใจอย่างมากอันเนื่องมาจากความสามารถในการงอกใหม่ได้ ซึ่งปัจจัยที่ทำให้เกิดการงอกใหม่นี้อาจจะนำมาประยุกต์ใช้ในการรักษาโรคที่มีความเกี่ยวข้องกับการเสื่อมสภาพทั้งในสัตว์เลี้ยงลูกด้วยนมและมนุษย์ โดยเฉพาะอย่างยิ่งในสัตว์เลี้ยงลูกด้วยนมที่มีความเสื่อมจาก endogenous pathway ได้

วัตถุประสงค์: เพื่อศึกษาผลการออกฤทธิ์ของสารสกัดโปรตีนปลิงทะเลขาว (*H. scabra*) ต่อกระบวนการแบ่งตัวของเซลล์ต้นกำเนิดมีเซนไคม์ (MSCs) จากรก

วัสดุและวิธีการ: นำปลิงทะเลขาวส่วนผนังลำตัว (BW) และส่วนอวัยวะภายใน (VI) มาทำการสกัดแยกโปรตีนด้วยสารละลายฟอสเฟต บัฟเฟอร์ซาลีน (PBS) และกรดอะซิติกที่ความเข้มข้น 0.1 โมลาร์ หลังจากนั้นนำสารสกัดที่ได้จากแต่ละส่วนมาทดสอบหาค่าความเป็นพิษและศึกษาผลของสารสกัดต่อการแบ่งตัวของ MSCs ที่แยกได้จากรกด้วยวิธี MTT assay และการนับจำนวนเซลล์ต้นกำเนิด

ผลการศึกษา: จากการแยกโปรตีนด้วยวิธี SDS-PAGE พบว่าการสกัดโดยใช้ PBS ความเข้มข้น 0.1 โมลาร์ ให้ปริมาณโปรตีนหลากหลายชนิดมากกว่าการสกัดด้วยกรดอะซิติกทั้งในส่วนของ BW และ VI ซึ่งโปรตีนส่วนใหญ่ที่พบใน BW จะมีน้ำหนักโมเลกุลประมาณ 38 และ 17 กิโลดาลตัน นอกจากนั้นยังพบว่าสารสกัดโปรตีนจากปลิงทะเลขาวที่ค่าความเข้มข้นต่ำไม่มีความเป็นพิษต่อ MSCs ในขณะที่ความเข้มข้นตั้งแต่ 0.01 ถึง 50 มคก./มล. สำหรับ BW และตั้งแต่ 0.01 ถึง 25 มคก./มล. สำหรับ VI จะพบว่ามีการกระตุ้นให้ MSCs มีการแบ่งตัวเพิ่มจำนวนมากขึ้นสอดคล้องกับผล growth kinetics ที่พบว่า BW ความเข้มข้นตั้งแต่ 0.1 ถึง 1 มคก./มล. สามารถเพิ่มอัตราการแบ่งตัวของเซลล์ได้มากกว่ากลุ่มควบคุม

สรุป: สารสกัดโปรตีนจากปลิงทะเลขาวมีผลต่อการแบ่งตัวของ MSCs ที่แยกได้จากรก อย่างไรก็ตามยังต้องมีการศึกษาเพิ่มเติมเกี่ยวกับคุณสมบัติของสารสกัด ซึ่งจากการทดลองนี้ทำให้เห็นได้ว่าสารสกัดนั้นสามารถช่วยกระตุ้นให้ MSCs เกิดการแบ่งตัวเพิ่มจำนวนสูงขึ้นได้