

<https://doi.org/10.15407/exp-oncology.2024.01.022>

**HANA RATNAWATI, TERESA LILIANA WARGASETIA,
LARISSA LARISSA, LIANA ALVITRI, KEANE BRYANT**

Faculty of Medicine, Maranatha Christian University, Bandung, Indonesia

* Correspondence: Email: Teresa.lw@med.maranatha.edu

HOLOTHURIA SCABRA METHANOL EXTRACT INHIBITS CANCER GROWTH THROUGH TGF- β /PI3K/P TEN SIGNALING PATHWAY IN BREAST CANCER MICE MODEL

Background. Molecules and cytokines can be targeted in cancer therapy. Transforming growth factor-beta (TGF- β) is a cytokine that acts on protein kinase receptors in the plasma membrane. The signaling pathway of TGF- β can trigger the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway, a signal transduction pathway important in cancer growth and development. However, this PI3K/AKT cascade can be inhibited by phosphatase and tensin homolog (*PTEN*) tumor suppressor genes. **Aim.** To determine the inhibitory effect of *Holothuria scabra* methanol extract (HSE) on breast cancer growth through the TGF- β /PI3K pathways and *PTEN* tumor suppressor gene on a breast cancer (BC) mice model. **Materials and Methods.** Female C57BL6 mice were subcutaneously injected with carcinogen DMBA 1 mg/kg body weight (BW) and fed a high-fat diet (HFD). Mice were randomly divided into five groups (n = 6): negative control (NC) administered with a standard diet, positive control (PC) administered with DMBA and HFD, and three treatment groups (T1, T2, and T3) treated with HSE doses of 0.33, 0.66, and 0.99 g/kg BW for 12 weeks. TGF- β concentration in the blood serum of mice was assessed by ELISA and the *PIK3CA* and *PTEN* gene expression by qRT-PCR. **Results.** The treatment with HSE resulted in a significant decrease in TGF- β concentrations in the blood sera of treatment groups T1 (35.31 \pm 17.33), T2 (43.31 \pm 17.42), and T3 (48.67 \pm 20.94) pg/mL compared to the PC group (162.09 \pm 11.60) pg/mL ($p < 0.001$). However, only HSE at a dose of 0.99 g/kg BW decreased the *PIK3CA* gene expression ($p = 0.026$), and at a dose of 0.66 g/kg BW increased the *PTEN* expression up to 4.93-fold. **Conclusion.** HSE is capable of inhibiting the TGF- β /PIK3CA pathway and increasing the *PTEN* gene expression.

Keywords: anticancer activity, breast cancer, *Holothuria scabra*, TGF- β /PIK3CA, *PTEN*.

Based on the data from the Global Cancer Observatory 2020, female breast cancer (BC) has surpassed lung cancer as the most commonly diagnosed cancer, with estimated 2.3 million new cases (11.7%) [1]. The data from the Indonesian Ministry of Health shows that BC ranks first as the leading

cause of death in cancer cases. The number of the new BC cases reached 65,858 (16.6%) of 396,914 cancer cases in Indonesia. Meanwhile, deaths have reached more than 22 thousand cases (9.6%), and more than 70% are at the advanced stage [2]. Studies are needed to develop new cancer therapy

Citation: Ratnawati H, Wargasetia TL, Larissa L, Alvitri L, Bryant K. *Holothuria scabra* methanol extract inhibits cancer growth through TGF- β /PI3K/P TEN signaling pathway in breast cancer mice model. *Exp Oncol.* 2024; 46(1): 22-29. <https://doi.org/10.15407/exp-oncology.2024.01.022>

© Publisher PH «Akademperiodyka» of the NAS of Ukraine, 2024. This is an open access article under the CC BY-NC-ND license (<https://creativecommons.org/licenses/by-nc-nd/4.0/>)

using one of Indonesia's marine natural resources sea cucumbers *Holothuria scabra*.

Many proteins and cytokines play a role in the pathogenesis of BC. In particular, loss of control of transforming growth factor-beta (TGF- β) signaling results in the increased cell proliferation and differentiation and decreased apoptosis [1]. TGF- β acts on the protein kinase receptors in the plasma membrane, which is essential in cancer cell proliferation [1, 2]. The signaling pathway of TGF- β can trigger the phosphatidylinositol-4,5-bisphosphate 3-kinase (PI3K) pathway, which plays a role in proliferation, cell growth, and cancer cell survival [3, 4]. The knockdown of the phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha (PIK3CA) gene with small-molecule inhibitors induced apoptosis and inhibition of the PI3K pathway signaling resulting in reduced cancer cell growth [5].

This PI3K/AKT cascade can be inhibited by the phosphatase tensin homolog (*PTEN*) located on chromosome 10q23.3. *PTEN* is a tumor suppressor gene frequently mutated in human cancer and has haplo-insufficient characteristics because it can disrupt the tumor suppressor function when it loses 50% of its function. A decreased *PTEN* expression is associated with poor prognosis and tumor resistance to therapy [6].

The *Holothuria scabra*, also known as a sea cucumber or "teripang" in Indonesia, is a marine invertebrate found in deep oceans and constituting an astounding biodiversity in Asia. This sea cucumber has long been used as food and medicine in Asia and the Middle East due to its synthesizing unique secondary metabolites [7].

Holothuria scabra, originating from the South China Sea, contains secondary metabolites of triterpene glycosides (saponins), which are scabra-sides A, B, and D. Those three metabolites show vigorous cytotoxicity against several cancer cells of different histogenesis, such as leukemia, lung cancer, hepatocellular carcinoma, colorectal cancer, and breast cancer [7].

Our previous study using a liquid chromatography-mass spectrometry (LC-MS) has shown that the methanol extract of *Holothuria scabra* isolated from the East Java coastal, Indonesia, contains three types of anticancer compounds, namely holothurin A holothurin B and holothurin B3 which inhibit tumor cell growth and increase the apoptosis [8].

Holothuria edulis, originally from Vietnam, also contains triterpene glycosides, holothurin A3 and A4. Both saponins are strongly cytotoxic to human epidermoid carcinoma and hepatoma cells. The bioactivity of sea cucumber triterpene glycosides is due to its membranolytic activity [9].

Those studies showed that active compounds from sea cucumbers have the potential to be anticancer candidates through various mechanisms. Therefore, we aimed to examine the anticancer mechanism of *Holothuria scabra* through an *in vivo* study using a BC mice model. Based on the role of TGF- β and PI3K pathways in the BC pathogenesis, this study aims to determine the inhibitory effect of *Holothuria scabra* on BC growth through the TGF- β /PI3K pathways and *PTEN* tumor suppressor gene in an *in vivo* model.

Materials and Methods

Breast cancer modeling in C57BL6 mice. This research on animal use has complied with all the relevant National Regulations and Institutional policies for the care and use of animals from the Faculty of Medicine, Maranatha Christian University No. 112/KEP/V/2021.

Female *Mus musculus* C57BL6 mice were bred in the iRATCo veterinary laboratory services, Bogor, Indonesia. Thirty female mice, aged 10–11 weeks, were assigned to five groups (n = 6), which were the negative control (NC) group (healthy mice fed with a standard diet), the positive control (PC) group (BC model), and three treatment groups (T1, T2, T3). Initially, PC, T1, T2, and T3 groups were fed with a high-fat diet (HFD with 57% fat supplied by the iRATCo laboratory) twice daily. On the 22nd day of the experiment, the mice were administered with 7,12-dimethylbenz[a]anthracene (DMBA) (Sigma — Aldrich, USA) 1 mg/kg BW, which had been dissolved in sesame oil (Sigma Aldrich, USA), injected subcutaneously 0.5 mL in one of the mice mammary [10, 11]. DMBA was injected 10 times every 2 days. Breast tumors were evaluated and palpated every week to indicate tumor growth. At the end of the experiment, blood was collected from the orbital veins in a volume of 0.5 mL, then serum was collected and stored at 4 °C for TGF- β assessment by an ELISA technique. All mice were sacrificed by cervical dislocation preceded by the Ketamine

List of primers used in the study

Gene	Forward primer	Reverse primer
<i>PIK3CA</i>	5'- TTATTGAACCAGTAGGCAACCG -3'	5'-GCTATGAGGCGAGTTGAGATCC -3'
<i>PTEN</i>	5'-TGGATTCGACTTAGACTTGACCT-3'	5'-GGTGGGTTATGGTCTTCAAAAGG-3'
<i>GAPDH</i>	5'- GAGAAACCTGCCAAGTATG -3'	5'- GGAGTTGCTGTTGAAGTC -3'

HCl (Dexa Medica, Indonesia) 100 mg/kg BW intraperitoneal injection.

The breast tumors were removed and weighted, paraffin-embedded tissue blocks were prepared, and the slides were stained with hematoxylin-eosin. The histopathological analysis was done using the Nottingham score. Three parameters, namely the tubular formation, nuclear pleomorphism, and mitotic activity, were assessed using a score of 1 to 3 for each parameter. The final score was based on the total score of these parameters (range from 3 to 9): a score of 3–5 as grade 1 (well differentiated), a score of 6–7 as grade 2 (moderately differentiated), and a score of 8–9 as grade 3 (poorly differentiated) [12].

The remaining mammary gland tissue samples were stored at –80 °C until use.

***Holothuria scabra* methanol extraction.** Fresh sea cucumber *Holothuria scabra* was collected from Malang Coastal, East Java, Indonesia. Sea cucumber identification was done based on the Food and Agriculture Organization of The United Nations Species Catalog for Fishery Purposes [13]. 20 g of sea cucumber were ground to a powder and then extracted with 500 mL of absolute methanol using the maceration method for 48 h. The solvent was filtered through Whatman filter paper No. 41, then evaporated using a rotary evaporator (Buchi R-114) at 50 to 60 °C.

Administration of HSE. 30 female mice were assigned to five groups (n = 6): the NC group, the PC group, and 3 treatment groups (T1, T2, and T3) treated with 3 various doses of *Holothuria scabra* methanol extract (HSE) 0.33 0.66 and 0.99 g/kg BW with oral gavage, once daily, for 12 weeks. The HSE was given on the 22nd day, simultaneously with the first DMBA injection.

TGF-β assessment by Sandwich-ELISA technique. Mouse TGF-β1 ELISA kit (Elabscience, USA) was used to evaluate TGF-β concentration, in which the microplates were pre-coated with an antibody specific for mouse TGF-β1. The assay procedure was carried out according to the

kit instructions. Samples of blood serum were added in triplicate to the microplate wells (100 μL for each well) and incubated for 90 min at 37 °C. The liquid was removed, and 100 μL of a biotinylated detection antibody working solution was added to each well and incubated for 1 h at 37 °C. The solution was aspirated from each well, 350 μL of wash buffer was added, soaked for 1 min, and then aspirated. This wash step was repeated 3 times. 100 μL of the HRP conjugate working solution was added to each well and incubated for 30 min at 37 °C. The wash process was repeated 5 times; 90 μL substrate reagent was added to each well and incubated for 15 min at 37 °C. The plate was protected from light. The enzyme-substrate reaction was terminated by adding 50 μL stop solution to each well. The optical density (OD) was measured by an ELISA reader at a wavelength of 450 nm. The OD value was proportional to the concentration of mouse TGF-β1.

qRT-PCR analysis of PIK3CA and PTEN gene expression. RNA extraction from BC tissue samples was performed using Genezol and continued with the qRT-PCR procedure using the Toyobo One-step RT-PCR kit with *GAPDH* as a housekeeping gene. The *PIK3CA*, *PTEN*, and housekeeping gene *GAPDH* primer sequences are listed in the Table:

2 μg RNA was reverse-transcribed into cDNA. Each reaction consisted of 60 ng cDNA as a template, 480 SYBR Green Master Mix, 0.4 μM forward primer, and 0.4 μM reverse primer, and the settings as follows: heating 5 min (95 °C), 40 cycles for 30 s (95 °C), 30 s (60 °C), and 60 s (72 °C).

Statistical analysis. Data analysis was performed using the Statistical Package of Social Science (SPSS ver. 25.0). Significant differences between the treatment groups were determined by the one-way ANOVA test and continued with post-hoc Tukey HSD for normal data and by non-parametric analysis, Kruskal — Wallis continued with the Mann — Whitney test for abnormal data. Differences between the treatment groups at $p = 0.05$ were considered statistically significant.

Results

The histological features of mammary gland samples are shown in Fig. 1, *a*, and the breast tumor weights are shown in Fig. 1, *b*. The mean breast tumor weight in the PC and T1, T2, and T3 groups was 0.63 ± 0.13 g, 0.27 ± 0.17 g, 0.30 ± 0.14 g, and 0.30 ± 0.17 g, respectively. The tumor weight reduction in the T1, T2, and T3 groups showed a significant difference ($p < 0.05$) compared to the PC group and between the HSE treatment groups. Therefore, HSE administration suppressed the breast tumor growth.

The results showed that a Nottingham score of the PC group (7.00 ± 1.26) was significantly different compared to the NC group (0.17 ± 0.41) with $p = 0.003$. This proves that a moderately differentiated breast cancer was formed in the DMBA and HFD treatment groups [14]. Data on the breast tumor weight and Nottingham score proved the development of breast tumors in this BC mice model.

TGF-concentration. The assessment of the TGF- β concentration in the blood serum of mice (Fig. 2) showed that this index was the highest in the PC group (162.09 ± 11.60 pg/mL) compared to the NC group (17.30 ± 3.35 pg/mL) ($p < 0.01$). HSE administration in 3 various doses decreased the TGF- β concentration: it was $35.31 \pm$

± 17.33 pg/mL 43.31 ± 17.42 pg/mL and 48.67 ± 20.94 pg/mL in T1, T2, and T3 groups respectively. These results indicated that HSE administration effectively reduced the TGF- β concentration by 69.97–78.21% but the reduction was not dose-dependent. Statistical analysis showed a highly significant difference between the PC group and all the treatment groups by this parameter but no significant difference ($p > 0.05$) between T1, T2, T3, and the NC group.

PIK3CA expression. It was reported that TGF- β can activate several intracellular signaling pathways, including the PI3K/Akt pathway [16]. Therefore, the *PIK3CA* gene expression was analyzed (Fig. 3).

The PC group showed the highest *PIK3CA* gene expression (1.050 ± 0.29) and a highly significant difference ($p = 0.0001$) compared to the NC group (0.270 ± 0.13). The administration of HSE in all doses reduced the *PIK3CA* gene expression in a dose-dependent manner, however, insignificantly groups T1 and T2 and significantly in group T3 (Fig. 3).

PTEN gene expression. The *PTEN* gene acts as a negative regulator of the PI3K/AKT pathway. We determined the effect of HSE administration on the *PTEN* gene expression (Fig. 4). As seen, the *PTEN* gene expression in the PC group (1.00 ± 0.12) was significantly lower ($p = 0.009$) com-

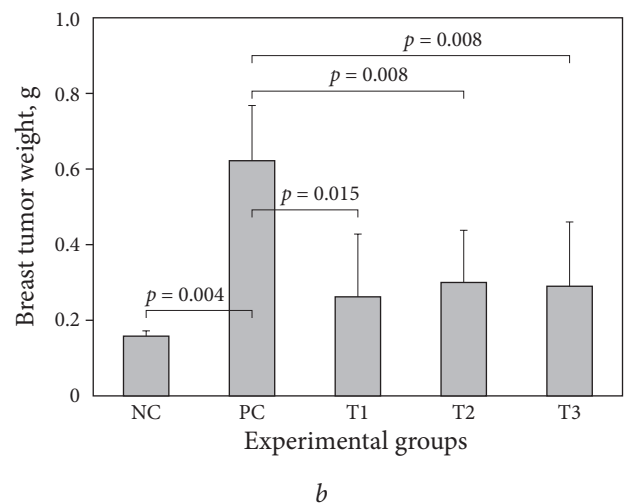
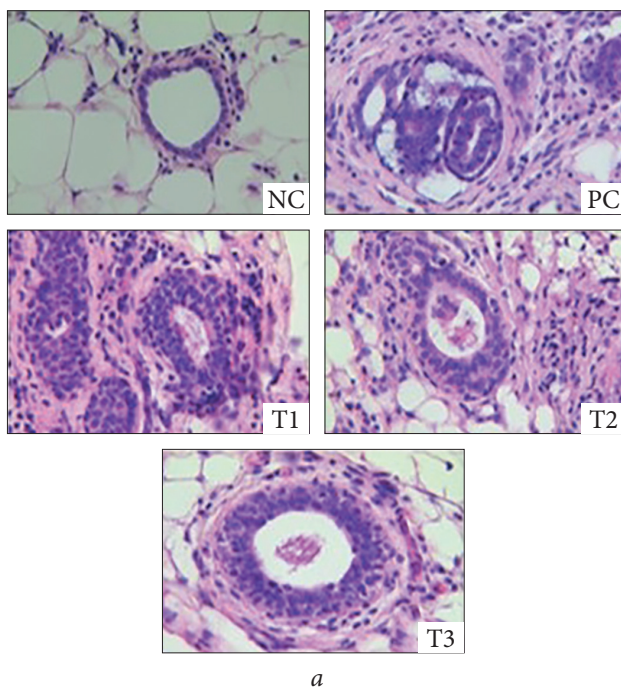


Fig. 1. Histological features (*a*) and breast tumor weight (*b*)

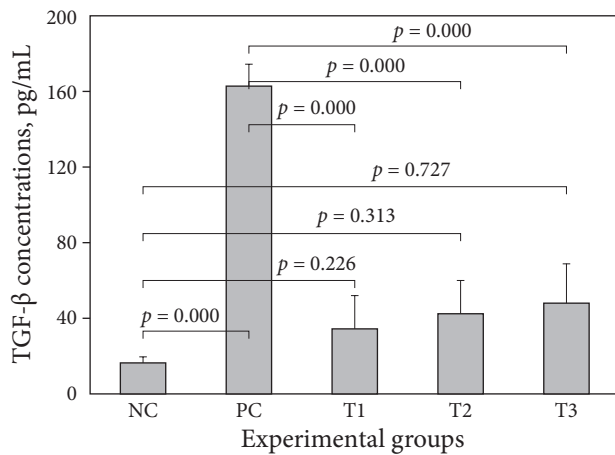


Fig. 2. TGF-β- concentration in the blood serum

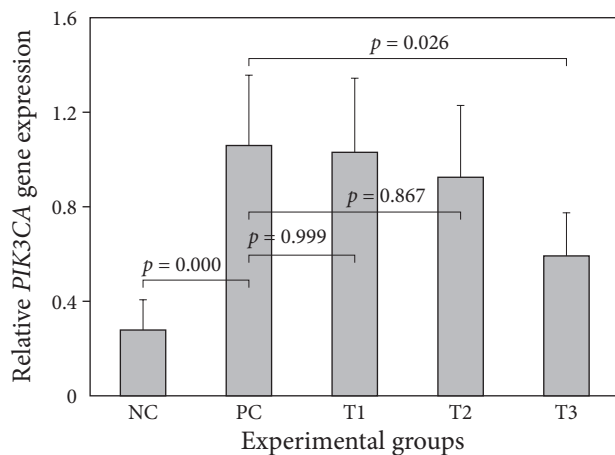


Fig. 3. PIK3CA gene expression in the groups of mice

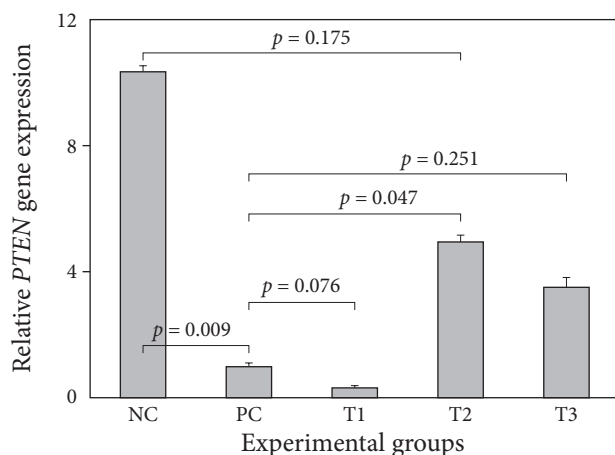


Fig. 4. PTEN gene expression in the groups of mice

pared to the NC group (10.28 ± 0.21). The HSE administration led to the increased *PTEN* gene expression in groups T2 and T3 (4.93 ± 0.21 and 3.49 ± 0.31 respectively) but not in group T1 (0.31 ± 0.08). The difference in *PTEN* gene expression between the T2 and NC groups was insignificant.

Discussion

Previous studies have proven the efficacy of DMBA and HFD used to construct a BC mice model. HFD accelerates cancer development [10, 11]. Increasing fat intake results in the leptin production by adipocytes. Gillespie's study [16] found that HFD significantly stimulates the early onset and increased the incidence of mammary tumors in DMBA-induced female mice. Leptin signaling is involved in DMBA-induced mammary carcinogenesis as leptin increases the secretion of several molecules related to BC [17, 18]. DMBA, a polycyclic aromatic hydrocarbon, is a carcinogen, acting through estrogen receptor signaling and the PI3K/Akt pathway. DMBA could induce BC in mice models, and 85% of such models exhibited a high level of *PI3KCA* and *PTEN* mutations with a significant activation of the PI3K/Akt pathway [18]. In our study, a combination of DMBA administration and HFD diet succeeded in obtaining BC development in mice.

In our previous study, the methanol extract of *Holothuria scabra* was analyzed using a liquid chromatography-mass spectrometry (LC-MS), and the results showed that *Holothuria scabra* contained three types of anticancer compounds, namely holothurin A, holothurin B, and holothurin B3. Based on *in silico* analysis, these holothurin compounds target proteins, which plays an essential role in the induction of apoptosis and tumor growth suppression [19]. In particular, they inhibited the growth of sarcoma-180 in mice and epidermal carcinoma cells *in vitro* [20].

The TGF-β assessment showed a significant effect of HSE administration on the TGF-β concentrations in the blood sera of mice. TGF-β plays a critical role in the tumor development. In the early phases, TGF-β frequently acts as a tumor suppressor by inducing the growth inhibition and apoptosis in premalignant cells. In contrast, tumor cells can resist its antimitogenic effects in the advanced phase, and TGF-β can switch into a tumor promoter [15, 21]. TGF-β can stimulate cancer development, interfere with cell cycle regulation, especially in the G1 phase, and induce tumor invasion and metastasis by promoting angiogenesis, epithelial-mesenchymal transition, and modifying the immune response [21, 22].

The TGF-β pathway is a classic membrane-to-nucleus signaling pathway with the involvement

of decapentaplegic (SMAD) proteins. The process begins with the attachment of the TGF- β ligand to receptors T- β R I and T- β R II, which then phosphorylate SMAD2/3 forming a complex with SMAD4, and then translocates into the nucleus and acts as a transcriptional factor toward the target genes, involved mainly in the growth arrest and apoptosis [21].

SMAD3 is known to be a target of the PI3K/Akt pathway. The activation of the PI3K/Akt pathway prevents the phosphorylation and activation of SMAD3, then the formation of the SMAD3/4 complex is inhibited. As a result, this molecular complex will not translocate into the nucleus to activate the apoptotic genes. The apoptotic process will be inhibited, and the cells will continue to proliferate. Thus, the PI3K/Akt pathway is an intracellular signaling pathway typically associated with cell proliferation and inhibition of apoptosis and is essential in the initiation and development of many malignancies through regulation of the TGF- β /SMAD4 pathway [23, 24].

In this study, HSE administration reduced the TGF- β concentrations in the blood sera of mice by 69.97%–78.21%. This decrease is thought to be due to the activity of holothurin as an anticancer compound of *Holothuria scabra* via inhibiting the TGF- β attachment to the T- β R I and T- β R II receptors, so that SMAD2/3 phosphorylation does not occur. As a result, the process of tumor cell apoptosis can take place, and this causes a reduction in the tumor weight by nearly twice in the HSE treatment groups.

As stated above, the TGF- β /SMAD4 pathway is also regulated by the PI3K/Akt pathway. One of the genes that play a role in its regulation is the *PIK3CA* gene [4]. Therefore, we also examined the *PIK3CA* gene expression level and revealed its dose-dependent decrease under conditions of HSE treatment.

According to Lu et al. [25], a bioactive peptide from sea cucumber has many health benefits, including antioxidant, immunomodulatory, and anticancer ones. Our previous bioinformatic study

with molecular docking has revealed that sea cucumber peptides have a potential for BC treatment. Two sea cucumber peptides (WPPNYQW and YDWRF peptides) bind to the active sites of PI3K and AKT1 with low binding affinity values and stable interactions to inhibit the PI3K/Akt pathway [26].

The tumor suppressor *PTEN* gene can counteract PI3K and inhibit the PI3K/Akt pathway, and *vice versa*, the loss of PTEN function results in the increased PIP3 level and the persistent activation of the PI3K effector [27].

In our study, the expression of the *PTEN* gene was found to be significantly lower in the PC group compared to the NC group. The low *PTEN* gene expression in BC mice models is in accordance with the statement that *PTEN* is one of the tumor suppressor genes most frequently inactivated in carcinogenesis. The loss or mutation in the *PTEN* gene is commonly detected in many primary and metastatic human cancers [6, 27].

The *PTEN* gene expression levels in the T2 and T3 groups were significantly higher compared to the PC group. The administration of HSE at a dose of 0.66 g/kg BW significantly increased the *PTEN* gene expression by 4.93 times.

To sum up, holothurins from *Holothuria scabra* methanol extract decreased the TGF- β concentration, inhibited the *PIK3CA* gene expression, and increased the *PTEN* gene expression in the murine BC model. Therefore, these compounds act through the TGF- β /PI3K and PTEN signaling pathways and are promising candidates for BC treatment.

Acknowledgment

This study was supported by the Universitas Kristen Maranatha, Bandung, Indonesia, under the B scheme grant in 2021 and 2022.

Conflict of Interest

All authors declare to have no conflict of interest regarding the article.

REFERENCES

1. Calone I, Souchelnytskyi S. Inhibition of TGF β signaling and its implications in anticancer treatments. *Exp Oncol*. 2012;34(1):9-16.
2. Khoshakhlagh M, Soleimani A, Binabaj MM, et al. Therapeutic potential of pharmacological TGF- β signaling pathway inhibitors in the pathogenesis of breast cancer. *Biochem Pharmacol*. 2019;164:17-22. <https://doi.org/10.1016/j.bcp.2019.03.031>
3. Fujio K, Komai T, Inoue M, et al. Revisiting the regulatory roles of the TGF- β family of cytokines. *Autoimmun Rev*. 2016;15(9):917-922. <https://doi.org/10.1016/j.autrev.2016.07.007>
4. Ghayad S, Cohen P. Inhibitors of the PI3K/Akt/mTOR Pathway: new hope for breast cancer patients. *Recent Pat Anticancer Drug Discov*. 2010;5(1):29-57. <https://doi.org/10.2174/157489210789702208>
5. Crawford RJ, Phommaly C, Tao Y, et al. PIK3CA and PIK3CB inhibition produce synthetic lethality when combined with estrogen deprivation in estrogen receptor-positive breast cancer. *Cancer Res*. 2009;69(9):3955-3962. <https://doi.org/10.1158/0008-5472>
6. Luongo F, Colonna F, Calapà F, et al. PTEN tumor-suppressor: the dam of stemness in cancer. *Cancers*. 2019;11(8):1076. <https://doi.org/10.3390/cancers11081076>
7. Pangestuti R, Ari Z. Benefit effects of functional sea cucumbers. *J Tradit Complement Med*. 2018;8(3):341-351. <https://doi.org/10.1016/j.jtcme.2017.06.007>
8. Wargasetia TL, Ratnawati H, Widodo N. Anticancer potential of Holothurin A, Holothurin B, and Holothurin B3 from the sea cucumber *Holothuria scabra*. *AIP Conf Proc*. 2020;2231. <https://doi.org/10.1063/5.0002552>
9. Hoang L, Le Thi V, Tran Thi Hong H, et al. Triterpene glycosides from the Vietnamese sea cucumber *Holothuria edulis*. *Nat Prod Res*. 2020;34(8):1061-1067. <https://doi.org/10.1080/14786419.2018.1548451>
10. Cranford TL, Velázquez KT, Enos RT, et al. Effects of high fat diet-induced obesity on mammary tumorigenesis in the PyMT/MMTV murine model. *Cancer Biol Ther*. 2019;3;20(4):487-496. doi:10.1080/15384047.2018.1537574
11. Kinoshita Y, Yoshioka M, Emoto Y, et al. Dietary effect of mead acid on DMBA-induced breast cancer in female Sprague-Dawley rats. *Int J Funct Nutr*. 2020;1(2):1. <https://doi.org/10.3892/ijfn.2020.7>
12. Simpson JF, Gray R, Dressler LG, et al. Prognostic value of histologic grade and proliferative activity in axillary node-positive breast cancer: results from the Eastern Cooperative Oncology Group Companion Study, EST 4189. *J Clin Oncol*. 2000;18(10):2059-2069. <https://doi.org/10.1200/JCO.2000.18.10.2059>
13. Purcell SW, Lovatelli A, González-Wangüemert M, et al. *Commercially Important Sea Cucumbers of the World*. Second edition. 2023. Rome, FAO. <https://doi.org/10.4060/cc5230en>
14. Wargasetia TL, Ratnawati H, Liana LK, et al. *Holothuria scabra* extract improves histopathological features and inhibits cancer growth through IL-6 and NF κ B signaling pathways in carcinoma mammae mice model. *Res J Pharmacogn*. 2024;11(2):1-9. <https://doi.org/10.22127/rjp.2023.417833.2233>
15. Xie F, Ling L, Van Dam H, et al. TGF-beta signaling in cancer metastasis. *Acta Biochim Biophys Sin (Shanghai)*. 2018;50(1):121-132. <https://doi.org/10.1093/abbs/gmx123>
16. Gillespie C, Quarshie A, Penichet M, et al. Potential role of leptin signaling in DMBA induced mammary tumors by non-responsive C57BL/6J Mice Fed a high-fat diet. *J Carcinog Mutagen*. 2012;3(132):20-23. <https://doi.org/10.4172/2157-2518.1000132>
17. Rosdianto AM, Kurniawan A, Gunadi JW, et al. DMBA-induced modulate estrogen receptors α and β breast cancer's animal model. *Maj Kedokt Bandung*. 2022;54(1):37-42. <https://doi.org/10.15395/mkb.v54n1.2479>
18. Abba MC, Zhong Y, Lee J, et al. DMBA induced mouse mammary tumors display high incidence of activating Pik3caH1047 and loss of function Pten mutations. *Oncotarget*. 2016;7(39):64289-64299. <https://doi.org/10.18632/oncotarget.11733>
19. Wargasetia TL, Ratnawati H, Widodo N. Anticancer potential of Holothurin A, Holothurin B, and Holothurin B3 from the sea cucumber *Holothuria scabra*. In: *AIP Conf Proc*. 2020;2231:040084. <https://doi.org/10.1063/5.0002552>
20. Khotimchenko Y. Pharmacological potential of sea cucumbers. *Int J Mol Sci*. 2018;19(5):1342. <https://doi.org/10.3390/ijms19051342>
21. Batlle E, Massagué J. Transforming growth factor- β signaling in immunity and cancer. *Immunity*. 2019;50(4):924-940. <https://doi.org/10.1016/j.immuni.2019.03.024>
22. Chen Y, Di C, Zhang X, et al. Transforming growth factor β signaling pathway: A promising therapeutic target for cancer. *J Cell Physiol*. 2020;235(3):1903-1914. <https://doi.org/10.1002/jcp.29108>
23. Song K, Wang H, Krebs TL. Novel roles of Akt and mTOR in suppressing TGF- β /ALK5-mediated Smad3 activation. *EMBO J*. 2006;25(1):58-69. <https://doi.org/10.1038/sj.emboj.7600917>
24. Zhao M, Mishra L, Deng CX. The role of TGF- β /SMAD4 signaling in cancer. *Int J Biol Sci*. 2018;14(2):111-123. <https://doi.org/10.7150/ijbs.23230>
25. Lu Z, Sun N, Dong L, et al. Production of bioactive peptides from sea cucumber and its potential health benefits: a comprehensive review. *J Agric Food Chem*. 2022;70(25):7607-7625. <https://doi.org/10.1021/acs.jafc.2c02696>

26. Wargasetia TL, Ratnawati H, Widodo N, et al. Bioinformatics study of sea cucumber peptides as antibreast cancer through inhibiting the activity of overexpressed protein (EGFR, PI3K, AKT1, and CDK4). *Cancer Informatics*. 2021;20:1-11. <https://doi.org/10.1177/11769351211031864>
27. Assinder SJ, Dong Q, Kovacevic Z, et al. The TGF- β , PI3K/Akt and PTEN pathways: established and proposed biochemical integration in prostate cancer. *Biochem J*. 2009;417(2):411-421. <https://doi.org/10.1042/BJ20081610>

Submitted: February 6, 2023

Х. Ратнаваті, Т.Л. Варгасетья, Л. Ларіса, Л. Алвітрі, К. Браїант
Факультет медицини, Християнський університет Мараната, Бандунг, Індонезія

TGF- β /PI3K/PTEN ОПОСЕРЕДКОВАНЕ ІНГІБУВАННЯ РОСТУ
РАКУ МОЛОЧНОЇ ЗАЛОЗИ У МИШЕЙ ЗА ДОПОМОГОЮ
МЕТАНОЛЬНОГО ЕКСТРАКТУ *HOLOTHURIA SCABRA*

Вступ. Цитокіни та фактори росту можуть бути використані в якості терапевтичних мішеней при різних типах раку. Трансформуючий фактор росту бета TGF- β є цитокіном, який зв'язується із рецепторами протеїнкінази на плазматичній мембрані. Він може активувати сигнальний шлях PI3K, який відіграє важливу роль у регуляції росту та розвитку злоякісних новоутворень. Однак каскад сигнального шляху PI3K/AKT може інгібуватися онкосупресорами, такими як PTEN. **Мета.** Визначити інгібуючу дію метанольного екстракту *Holothuria scabra* (HSE), опосередковану сигнальним шляхом TGF- β /PI3K та PTEN, на ріст раку молочної залози в мишей, індукованого 7,12-диметилбенз[а]антраценом (ДМБА) і дієтою з високим вмістом жирів. **Матеріали та методи.** Самкам мишей C57BL6 підшкірно вводили канцероген ДМБА у дозі 1 мг/кг маси тіла і годували кормом із високим вмістом жиру з метою індукції раку молочної залози. Мишей випадковим чином розділяли на п'ять груп (n = 6): негативний контроль (НК) із стандартною дієтою, позитивний контроль (ПК) — індукований рак молочної залози та три групи тварин із раком молочної залози, які отримували терапію (T1, T2, T3) у вигляді HSE у дозах 0,33, 0,66 та 0,99 г/кг маси тіла протягом 12 тижнів. Дослідження концентрації TGF- β проводили методом ELISA, а експресію генів *PIK3CA* та *PTEN* визначали методом qRT-PCR. Дані проаналізовано з використанням однофакторного дисперсійного аналізу ANOVA. **Результати.** Концентрація TGF- β у тварин, що отримували терапію T1 (35,31 \pm 17,33), T2 (43,31 \pm 17,42) і T3 (48,67 \pm 20,94), значно відрізнялася від групи ПК (162,09 \pm 11,60) з $p < 0,001$. HSE у всіх використаних дозах приводив до зниження рівня TGF- β , незалежно від дози, проте лише при використанні HSE в дозі 0,99 г/кг маси тіла відмічено зниження експресії *PIK3CA* ($p = 0,026$). HSE в дозі 0,66 г/кг маси тіла приводив до збільшення експресії *PTEN* у 4,93 рази. **Висновок.** Завдяки здатності інгібувати сигнальний шлях TGF- β /PIK3CA та стимулювати експресію *PTEN*, HSE може стати основою для подальшого розроблення засобів для лікування раку молочної залози.

Ключові слова: протипухлинна дія, рак молочної залози, *Holothuria scabra*, TGF β /PIK3CA, PTEN.