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Research Article
Triterpenes in the Ethanol Extract of *Poria cocos* Induce Dermal Papilla Cell Proliferation

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Abstract

Background and Objective: Many people are affected by hair loss, although certain drugs can contribute to hair growth. Developing a new medical therapy for hair loss will be helpful for improving people's lives. This study was aimed to investigate the effects of *Poria cocos* extract on human follicle dermal papilla cells (HFDPCs) to identify potential compounds for hair growth. **Materials and Methods:** The WST-1 assay and bromodeoxyuridine tests were used to evaluate cell proliferation. The cell cycle was examined by flow cytometry. The expression levels of cell cycle-associated proteins and major mitogen-activated protein kinases were determined by western blot. **Results:** *Poria cocos* extract was found to increase the cell viability and induce cell proliferation of HFDPCs at concentrations ranging from 25-100 μg mL^{-1}. The expression levels of cell cycle-associated proteins were increased in *P. cocos* extract-treated HFDPCs. Moreover, increased expression levels of p-AKT and p-ERK were found in *P. cocos* extract-treated HFDPCs. Each triterpene in *P. cocos* extract also exhibited a similar effect on HFDPCs proliferation. In addition, blockade of AKT kinase but not ERK abolished *P. cocos* extract-induced HFDPC proliferation. **Conclusion:** The present results suggested that the AKT signaling pathway is involved in *P. cocos* extract-induced cell proliferation of HFDPCs.

Key words: *Poria cocos* extract, triterpene, human follicle dermal papilla cells, hair loss, cell proliferation, hair growth, cell viability

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Poria cocos, also called Fu-ling in Chinese, is a fungus belonging to the Polyporaceae family. It has a wide spectrum of pharmacological activities and has a long history of use as a Chinese traditional medicine[1]. *Poria cocos* has been shown to be able to promote antitumor[2-5], antioxidant[6], antibacterial[7], antihyperglycemia[8,9] and anti-inflammatory[10,11] activities. The bioactive properties in *P. cocos* constituents include polysaccharides, triterpenoids, fatty acids, sterols and enzymes. Among these constituents, polysaccharides and triterpenoids have been reported as the main bioactive components in *P. cocos*[12-15].

Tumulosic acid (TA), a triterpenoid found in *P. cocos*, has been reported to induce lung cancer cell death by down regulating Bcl-2 expression and activating the caspase cascade[2]. Three triterpenes, including pachymic acid (PA), dehydropachymic acid (DPA) and polyporenic acid C (PAC), have been shown to suppress the proliferation of human pancreatic cancer cell lines and inhibit cell invasion via down regulation of matrix[12] metalloproteinase-7. Six lanostane-type triterpenes isolated from *P. cocos* exhibited inhibitory effects on phorbol ester-induced Epstein-Barr virus early antigen activation in Raji cells and could suppress the formation of skin tumors[5].

Hair loss (baldness) refers to a loss of hair from the scalp or can be severe enough to affect the entire body. Common baldness types include patterned hair loss, alopecia areata, telogen effluvium and patterned hair loss is the most common cause of hair loss found after puberty[16]. Although the causes of hair loss are still not completely understood, infection by fungi, several medications, or immune disorders could cause temporary or permanent hair loss[17].

The hair follicle growth cycle consists of 3 phases: A long growing anagen phase, a regressing catagen phase and a short resting telogen phase[18]. After the resting telogen phase, the hair falls out and a new growing anagen phase begins. Too many hairs falling out or a disruption at the growing anagen phase would lead to abnormal hair loss. The telogen-to-anagen transition occurs near the dermal papilla. These stem cells proliferate rapidly and amplified daughter cells from the new hair follicle[19]. Therefore, dermal papilla cells play a crucial role in the hair growth cycle and could be the target cells used for studying the cellular mechanisms organizing the hair follicle.

The aim of this study was to investigate the benefits of alternative natural compounds in the growth of human follicle dermal papilla cells (HFDPCs) as potential therapeutics for hair loss. In addition, the underlying mechanisms involved in the effects of the *P. cocos* extract on the cell proliferation of HFDPCs were also determined.

MATERIALS AND METHODS

This study was performed at the Core Laboratory of the Buddhist Tzu Chi General Hospital Taipei Branch (New Taipei City, Taiwan) and Radiation Biology Core Laboratory, Chang Gung Memorial Hospital (Taoyuan, Taiwan) from January, 2018-September, 2019.

Preparation of an ethanol extract of *P. cocos* (PEE): Dried sclerotia of *P. cocos* was purchased from Sun Ten Pharmaceutical Co., Ltd. (Taiwan). About 10 g of *P. cocos* extract was extracted with 95% ethanol by sonication. The extract was then filtered using filter paper and stored at -20°C for further use. The dehydrotumulosic acid (DTA), TA and PA were obtained from Carbosynth (Berkshire, UK). The 3-epidehydrotumulosic acid (EDTA) and PAC were purchased from ChemFaces Biochemical Co., Ltd. (Hubei, USA).

Cell culture: HFDPCs, which were isolated from female donors, were purchased from PromoCell (Heidelberg, Germany). Cells were maintained in low serum cell culture medium (PromoCell), which is optimized for the maintenance of HFDPCs. The 4th-10th HFDPC population doublings were used in this study. There were no significant changes in the morphology or alkaline phosphatase activity of the 10th population doublings of HFDPCs.

Cell viability assay: The Cell viability assay was performed as described previously by Tsai *et al.*[20]. In brief, HFDPCs were seeded in 24-well plates in triplicate with a cell density of 5×10^4 cells/well. HFDPCs were serum starved for 24 h, followed by incubation in cultured medium supplemented with 0.5% fetal calf serum and various concentrations of PEE or 50 μM triterpenes found in PEE for 24 h. One hundred microliters of colorimetric WST-1 reagent (Roche Diagnostics, Mannheim, Germany) was added to the cells and then they were incubated at 37°C for an additional 4-6 h. The cultured medium was then transferred to a 96-well plate and the absorbance at 450 and 650 nm was determined in a multifunctional microplate reader (Infinite F200, Tecan, Durham, NC, USA).

Bromodeoxyuridine (BrdU) cell proliferation assay: The incorporation of BrdU were used to evaluate HFDPCs proliferation[20]. Briefly, HFDPCs were seeded in 24-well plates in triplicate with a cell density of 3×10^4 cells/well. HFDPCs were serum starved for 24 h, followed by incubation in cultured medium supplemented with 0.5% fetal calf serum and various concentrations of PEE or 50 μM triterpenes found in PEE for 48 h. Cells were incubated with 10 μM BrdU labeling solution (Roche Diagnostics) at 37°C for an additional 12 h. The culture medium was removed and cells were fixed and denatured by adding FixDenat for 30 min. After removing the fixing solution, cells were then incubated with an anti-BrdU-POD antibody at 25°C for 90 min. The mixtures of antibodies were removed, followed by incubation with tetramethylbenzidine substrate. The substrate mixtures were incubated at 25°C until color development was sufficient for detection. The absorbance of each well was measured in a multifunctional microplate reader at 370 and 492 nm.

Cell cycle analysis: The cell cycle analysis for HFDPCs was performed as described previously by Tsai *et al.*[20]. In brief, HFDPCs were seeded in 6-cm dishes with a cell density of 3×10^5 cells/dish. HFDPCs were serum starved for 24 h, followed by incubation in cultured medium supplemented with 0.5% fetal calf serum and various concentrations of PEE for 48 h. Cells were washed with 1×PBS and harvested by centrifugation. After centrifugation, the cell pellets were vigorously vortexed while slowly adding ice-cold 80% ethanol. After fixation overnight at -20°C, the cells were then stained with 500 μg mL^{-1} propidium iodide (Sigma-Aldrich, St. Louis, MO, USA) containing 0. 1% (v/v) Triton X-100 solution at 25°C for 30 min. Aggregates were removed with 30 μm pore size filters and the staining of cells was then analyzed by flow cytometry (Cytomics FC 500; Becton Dickinson, Franklin Lakes, NJ). Approximately 5,000 events were collected from each sample and the percentage of the cell cycle phase distribution in each sample was then analyzed and processed with Multicycle for Windows software (Becton Dickinson).

Western blotting: Protein expression analysis were performed as described previously Tsai *et al.*[20]. HFDPCs were seeded in 6 cm dishes with a cell density of 3×10^5 cells/dish. HFDPCs were serum starved for 24 h, followed by incubation in cultured medium supplemented with 0.5% fetal calf serum and various concentrations of PEE or 50 μM triterpenes found in PEE for 24 h. Cells were washed three times with 1×PBS and lysed with RIPA lysis buffer (20 mM Tris-HCl at pH 7.5, 100 mM NaCl, 1% Nonidet P40, 100 μM Na_3VO_4, 50 mM NaF, 30 mM sodium pyrophosphate) containing 1×complete protease inhibitor cocktail (EDTA-free) (Roche) at 4°C for 30 min. Cell lysates were centrifuged to remove debris and proteins in the supernatant were collected and stored at -80°C until use. About 20-50 μg of total protein from each sample was separated on 12% polyacrylamide gels and the resolved protein samples were then transferred to polyvinylidene difluoride membranes. After blocking with 1×PBS containing 5% non fat dry milk and 0.1% Tween-20, membranes were hybridized with the following primary antibodies at 4°C for 12 h and then incubated with horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit antibodies at room temperature for 2 h. An ECL kit (Amersham, Bucks, UK) was used to detect the substrate reaction and the band for target proteins was detected with a ChemiDoc™ XRS+System (Bio-Rad Laboratories, Hercules, CA). Antibodies used for detection of Cyclin D1 (Cell Signaling Technology, Beverly, MA, USA), Cyclin E1 (Cell Signaling Technology), Cyclin A (Cell Signaling Technology), Cdk2 (Cell Signaling Technology), Cdk4 (Cell Signaling Technology), Cdc2 (Cell Signaling Technology), p-AKT (Cell Signaling Technology), AKT (Cell Signaling Technology), p-p44/p42 ERK (Cell Signaling Technology), p44/p42 ERK (Cell Signaling Technology), p-JNK (Cell Signaling Technology), JNK (Cell Signaling Technology), p-p38 (Cell Signaling Technology), p38 (Cell Signaling Technology) and Actin (Sigma-Aldrich) are indicated.

Statistical analysis: All experiments were performed in triplicate. All statistical analysis were carried out using one-way ANOVA with Dunnett's *post hoc* test. Data are presented as the mean±standard deviation. A p-value <0.05 indicated statistical significance.

RESULTS

PEE increased the viability of HFDPCs: In order to evaluate the effect of PEE on HFDPC growth, the viability of HFDPCs treated with different doses of PEE was determined by WST-1. PEE increased HFDPC viability in a dose-dependent manner, with a maximal value in HFDPCs treated with 100 μg mL^{-1} PEE (Fig. 1a). In addition, the effect of PEE on the cell death of HFDPCs was also examined, which was determined by lactate dehydrogenase release. No apparent cell death was observed in HFDPCs treated with different doses of PEE. Since PEE could increase HFDPC viability, the effect of PEE on the cell proliferation of HFDPCs was then examined, as determined by the BrdU incorporation assay. As shown in Fig. 1b, HFDPC proliferation was significantly increased in cells treated with

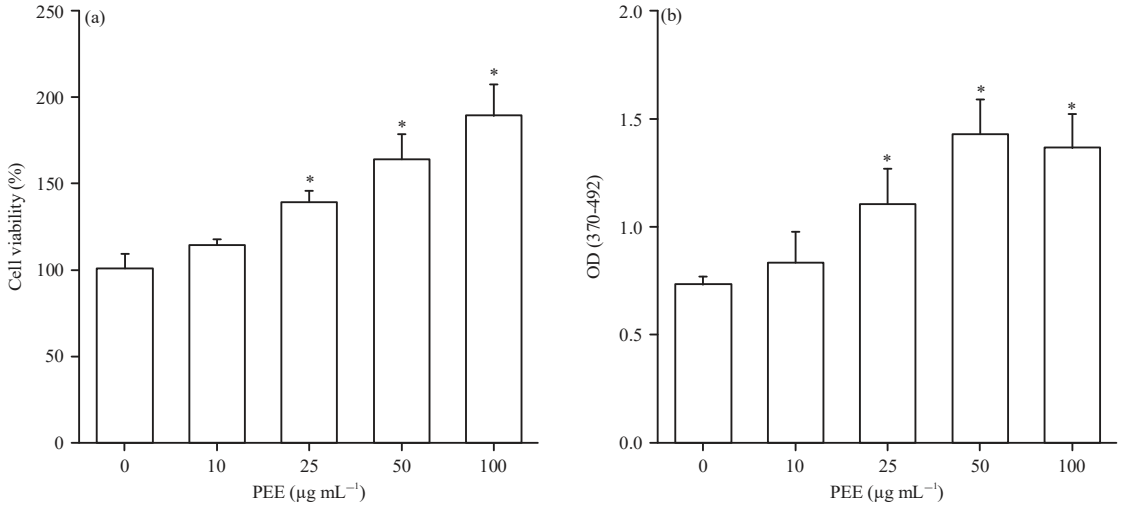

Fig. 1(a-b): Effect of PEE on the (a) Viability and (b) Cell proliferation of HFDPCs

HFDPCs were treated with the indicated concentrations of PEE for 24-48 h, cell viability and cell proliferation were analyzed by WST-1 and BrdU assays, respectively, data were expressed as Mean±SD, *p<0.05 compared with control group, PEE: An ethanol extract of *P. cocos*

25-100 μg mL^{-1} PEE, with a maximal capacity of cell proliferation in HFDPCs treated with 50 μg mL^{-1} PEE. The cell cycle in PEE-treated HFDPCs was further analyzed. As shown in Fig. 2, treatment with PEE could dose-dependently increase the proportion of HFDPCs in S phase (from 10.6-25%) and reduce the proportion of cells in G1 phase (from 83.1-69.2%) when compared to that in the control group.

PEE increased cell cycle-associated proteins in HFDPCs: Because PEE could increase the proportion of cells in S phase, the expression levels of cell cycle-associated proteins in PEE-treated HFDPCs were then detected. Cyclin D1/cdk4 are the first cyclin- and cyclin-dependent kinases(cdk) to be upregulated in G1 phase. The level of cyclin D1 and cdk4 were increased in HFDPCs treated with 25-100 μg mL^{-1} PEE (Fig. 3a). Cyclin E1 and cdk2 are considered cell cycle-associated proteins involved in the G1-S transition. Both the expression levels of cyclin E1 and cdk2 increased in a dose-dependent mannerin HFDPCs treated with PEE (Fig. 3a). Cyclin A and cdc2, believed to be involved in the G2/M transition, also increased dose-dependently in HFDPCs following PEE treatment (Fig. 3a).

To investigate the possible mechanism underlying PEE-induced HFDPC proliferation and the increase in expression of cyclins and cdks, the levels of 4 major mitogen-activated protein kinases were determined. Treatment with high doses of PEE (50-100 μg mL^{-1}) led to a slight decrease in the level of p-JNK, while the levels of p-p38 were not affected by PEE (Fig. 3b). In contrast, the levels of p-AKT and p-p42/p44 ERK increased in a concentration-dependent manner in HFDPCs treated with PEE (Fig. 3b). These observations suggest that PEE-induced HFDPC proliferation might be mediated by the upregulation of cell cycle-associated proteins via activation of the AKT and p42/p44 ERK pathways.

Triterpenes induced HFDPC proliferation: PEE contains approximately 60% triterpenoids and 40% lipid[2] and these triterpenoids contain TA, DTA, PAC, EDTA and PA. In order to evaluate whether PEE-induced HFDPC proliferation is associated with these triterpenoids, HFDPCs were treated with each component found in PEE. As shown in Fig. 4a, treatment with all of the triterpenoids used in this study significantly increased cell viability. Furthermore, the cell proliferative ability was significantly increased in HFDPCs treated with these triterpenes when compared to that of the control-treated group (Fig. 4b).

Triterpenes increased cell cycle-associated proteins and p-AKT in HFDPCs: To investigate whether HFDPC proliferation induced by triterpenes found in PEE also led to increased expression of cell cycle-associated proteins, the cyclins and cdks were determined in cell lysates collected from triterpene-treated HFPDCs. All of the triterpenes used in this study exerted similar effects as PEE in HFDPCs to increase the expression levels of cyclins/cdks (Fig. 5a).

The effect of triterpenes found in PEE on the expression levels of AKT and p42/p44 ERK was also examined. As shown in Fig. 5b, treatment with PEE and triterpenes found in PEE increased the level of p-AKT. The level of p-p42/p44 ERK was

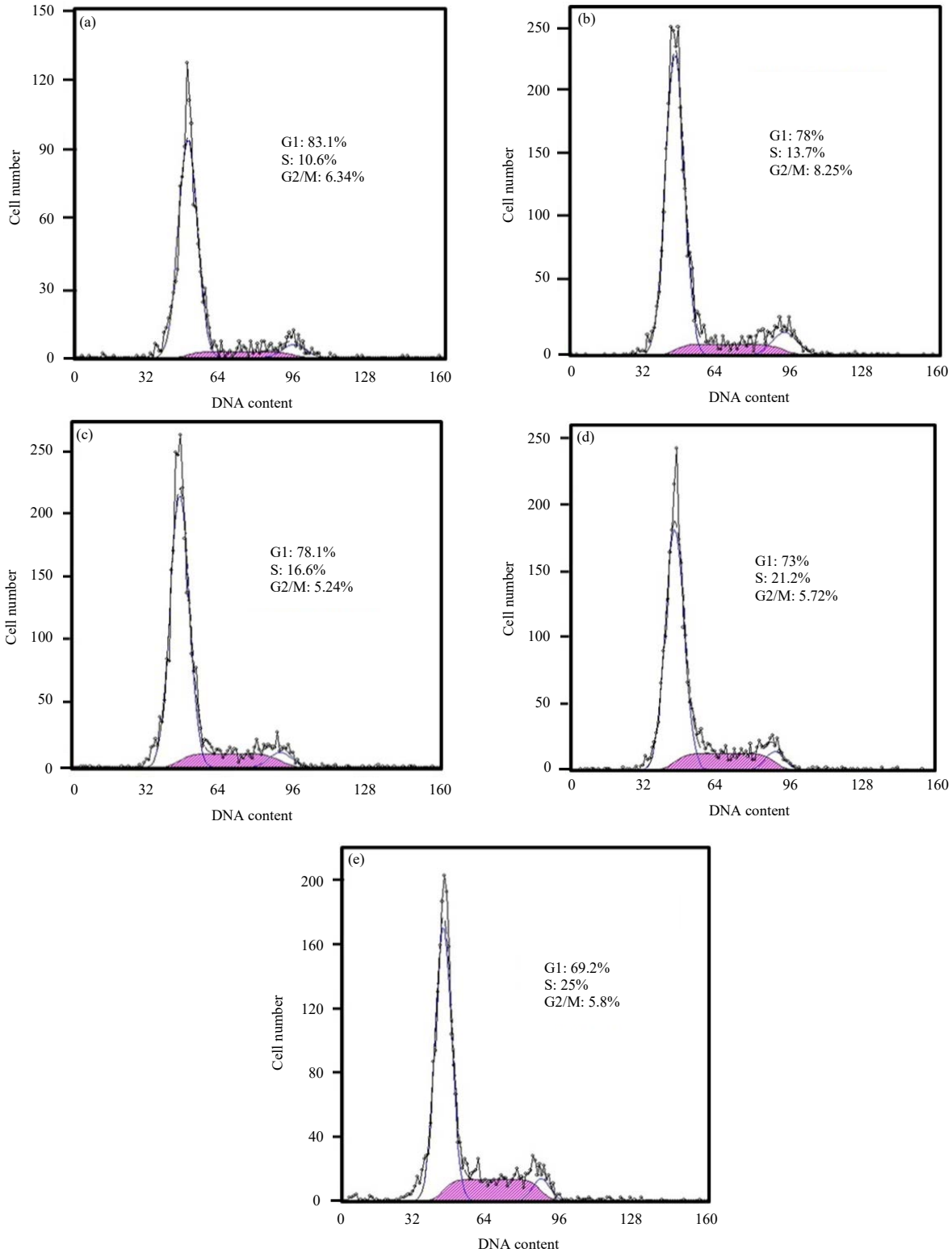

Fig. 2(a-e): Flow cytometric analysis was performed to evaluate the cell cycle in PEE-treated HFDPCs, (a) Control, (b) 10 μg mL^{-1} PEE, (c) 25 μg mL^{-1} PEE, (d) 50 μg mL^{-1} PEE and (e) 100 μg mL^{-1} PEE

Flow cytometric analysis of cell cycle parameters following 48 h of treatment with indicated concentrations of PEE compared with untreated control cells, PEE: An ethanol extract of *P. cocos*

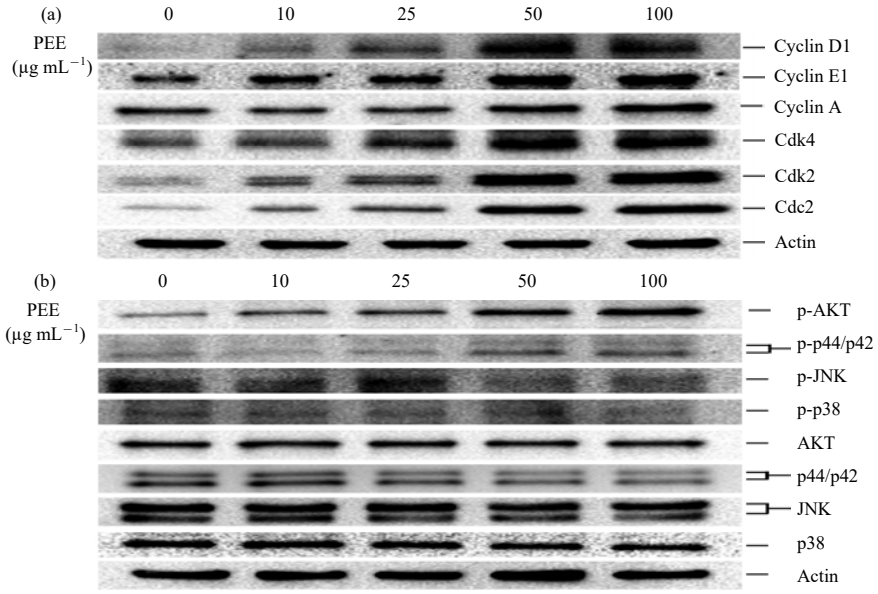

Fig. 3(a-b): Levels of (a) Cell cycle-associated proteins and (b) Four mitogen-activated protein kinases in PEE-treated HFDPCs were detected by western blot

HFDPC were treated with the indicated concentrations of PEE for 24 h, expression levels of cell-associated proteins and mitogen-activated protein kinases were evaluated, PEE: An ethanol extract of *P. cocos*

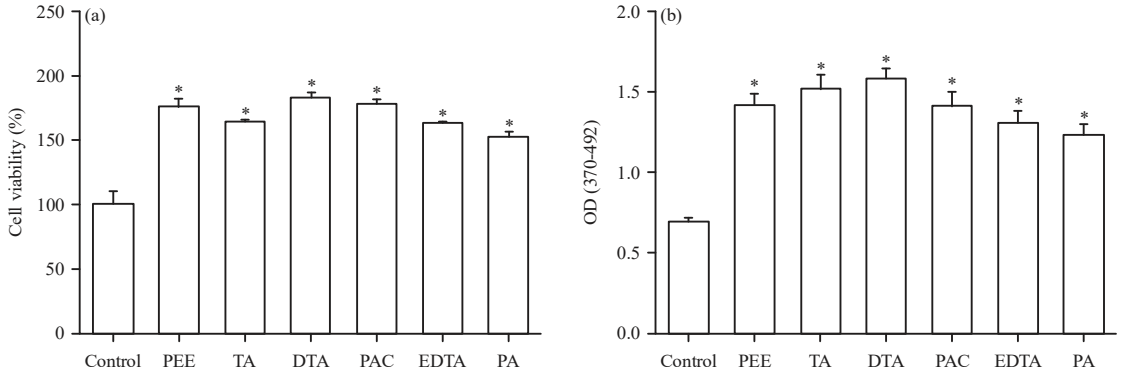

Fig. 4(a-b): Effect of each triterpene in PEE on the (a) Viability and (b) Cell proliferation of HFDPCs

HFDPCs were treated with the 50 μg mL^{-1} PEE or 50 μM indicated triterpene for 24-48 h, data were expressed as Mean±SD, *p<0.05 compared with control group, PEE: An ethanol extract of *P. cocos*, TA: Tumulosic acid, DTA: Dehydrotumulosic acid, PAC: Polyporenic acid C, EDTA: 3-epidehydrotumulosic acid, PA: Pachymic acid

also increased in HFDPCs treated with PEE, DTA and PAC when compared to the control-treated group. No apparent change in the level of p-p42/p44 ERK was observed in HFDPCs treated with TA, EDTA and PA (Fig. 5b).

AKT kinase inhibitor abrogated PEE-mediated HFDPC proliferation: To further confirm the possible association between activation of mitogen-activated protein kinases and PEE-mediated HFDPC proliferation, inhibitors for AKT kinase and p42/p44 ERK were used. The cell viability was decreased in HFDPCs treated with AKT kinase inhibitor and PD98059 (p42/p44 ERK inhibitor) when compared to the viability with control treatment. PD98059 did not block triterpenes in the PEE-mediated increase in cell viability. In contrast, the increased cell viability induced by triterpenes in PEE was completely abolished in the HFDPCs treated with AKT kinase inhibitor (Fig. 6a).

Similarly, cell proliferation was decreased in HFDPCs treated with AKT kinase inhibitor or PD98059. The decrease in cell proliferation was similar in control and triterpene-treated

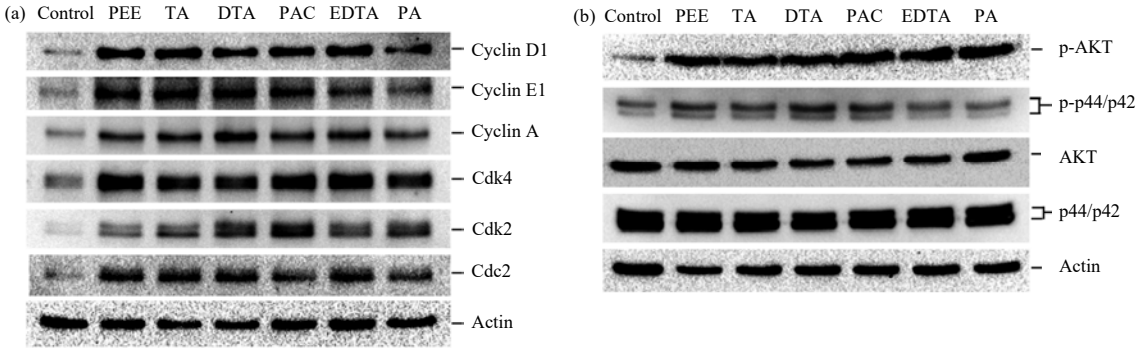

Fig. 5(a-b): Levels of (a) Cell cycle-associated proteins and (b) p-AKT and p-ERK in each triterpene in PEE-treated HFDPCs were detected by western blot

HFDPC were treated with 50 μg mL^{-1} PEE or 50 μM indicated triterpene for 24 h, expression levels of cell-associated proteins, AKT and p42/p44 were evaluated, PEE: An ethanol extract of *P. cocos*, TA: Tumulosic acid, DTA: Dehydrotumulosic acid, PAC: Polyporenic acid C, EDTA: 3-epidehydrotumulosic acid, PA: Pachymic acid

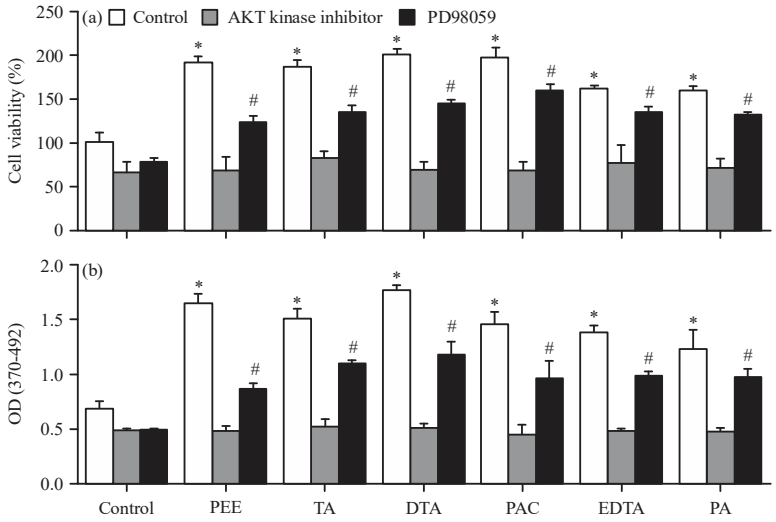

Fig. 6(a-b): Effect of the AKT kinase inhibitor and PD98059 on (a) Viability and (b) Cell proliferation in PEE-treated HFDPCs

HFDPCs were treated with 50 μg mL^{-1} PEE or 50 μM indicated triterpene in the presence or absence of the AKT1/2 kinase inhibitor (5 μM) or PD98059 (50 μM) for 24-48 h, *p<0.05 compared with control group, #p<0.05 cells were treated with PEE or triterpene and PD98059 compared to cells were treated with PD98059, PEE: An ethanol extract of *P. cocos*, TA: Tumulosic acid, DTA: Dehydrotumulosic acid, PAC: Polyporenic acid C, EDTA: 3-epidehydrotumulosic acid, PA: Pachymic acid

HFDPCs combined with PD98059, indicating that ERK was not the candidate protein involved in PEE-mediated HFDPC proliferation. However, PEE-induced cell proliferation was completely blocked in the HFPDCs treated with the AKT kinase inhibitor (Fig. 6b). Therefore, the increase in the number of viable cells and the proliferation ability of HFDPCs induced by PEE occurred through the AKT signaling pathway.

DISCUSSION

The present study investigated the effects of *P. cocos* extract on the growth of HFDPCs. Results showed that PEE significantly increased the proliferation of HFDPCs. The levels of cell cycle-associated cyclins and cdks were also induced by PEE. In addition, both the activity of p42/p44 ERK and AKT was induced by PEE in HFDPCs. Furthermore, blockade of AKT but not ERK abolished PEE-induced HFDPC proliferation, indicating the role of the AKT pathway in PEE-mediated HFDPC growth.

It will be helpful to study the effective compounds in *P. cocos* extract to further demonstrate its promotion of cell proliferation of HFDPCs. However, there is little information in the literature regarding the binding targets for the compounds in *P. cocos* extract. Triterpenoids (bodinone,

bodinone glycoside and D-sorbitol) in *Schefflera bodinieri* selectively bind to muscarine receptors with IC_{50} values of 0.91-3.24 μM, while trisaccharide can bind to Ca^{2+} channels and 5HT-2 receptors with IC_{50} values of 8.03 and 3.81 μM, respectively. Additionally, dopamine receptors could be bound by bodirin A and all of the receptors for 5HT1C, 5HT2, opiate, beta-adrenergic and histamine 1 could be affected by bodirin A, bodinitin A and the trisaccharide[21]. It is still not clear whether triterpenoids or trisaccharides could be agonists or antagonists of these receptors, even if these receptors could be bound by triterpenoids or trisaccharides. The mechanism of cell proliferation of HFDPCs induced by *P. cocos* extract, which is mediated through these receptors bound by triterpenoids in *P. cocos*, needs further investigation.

Although PEE has no effect on the cell death of HFDPCs, PEE has been reported to exhibit proapoptotic activity in cancer cells[2,14]. Elevated caspase activity was observed in HL60 and leukemia A549 lung cancer cells treated with PEE or TA. However, *P. cocos* extract protected PC12 neuron cells or LLC-PK1 kidney tubular epithelial cells from cell death induced by β-amyloid or cisplatin, respectively[22,23]. Additionally, a previous study showed that the levels of cell cycle-associated proteins were decreased in A549 cells treated with *P. cocos* extract[2]. These results suggested that the different results from cancer cells and normal cells may be caused by these receptors/targets bound by compounds in *P. cocos* extract present in different cell lines.

Based on the causes of hair loss, the treatment for hair loss can be targeted to the regulation of immune response, hormone production and hair regrowth. Some medications were made for the treatment of hair loss, including minoxidil and finasteride. Finasteride is used to treat male pattern baldness because the 5α-reductase inhibitor finasteride inhibits the formation of androgen from testosterone[24]. Some naturally derived compounds also exhibited similar effects on hair follicles via 5α-reductase[25]. The K_{ATP} opener minoxidil could cause hyperpolarization of cell membranes[26], subsequent broadening of blood vessels and the allowance of more blood and nutrients into the hair follicles. The influx of blood induced by minoxidil may facilitate the telogen-anagen transition in hair follicles[27,28]. Ebastine, a histamine receptor inhibitor, has been shown to exert favorable effects on hair growth via immunoregulation and hair regrowth[20,29].

This study indicated that the *P. cocos* extract could directly induce HFDPC proliferation via the AKT signaling pathway. The target of Chinese traditional medicine is different from minoxidil for hair loss. Moreover, curing hair loss with the combination of treatments targeting blood influx and hair growth might be further considered.

CONCLUSION

The results of this study demonstrate that the *P. cocos* extract could induce HFDPC proliferation. The levels of cell cycle-associated proteins, activated p42/p44 ERK and activated AKT were upregulated by *P. cocos* extract in HFDPCs. Similar effects as *P. cocos* extract exhibited by triterpenes in *P. cocos* extract on HFDPC proliferation indicate a role for triterpenes in *P. cocos* extract-regulated HFDPC growth.

SIGNIFICANCE STATEMENT

This study discovered that the *P. cocos* extract could directly induce HFDPC growth, which can be beneficial for curing hair loss. The current study also demonstrated the potential capacity of naturally derived compounds to enhance hair follicle cell regrowth. This study also suggested that the combination of the drug currently available for treating balding that targets blood influx combined with *P. cocos* extract might improve the success of the telogen-anagen transition to cure hair loss. This study will help the researcher to uncover the critical areas of dermatology that many researchers were not able to explore. Thus, a new theory on dermatology may be arrived at.

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REFERENCES

1. Wang, Y.Z., J. Zhang, Y.L. Zhao, T. Li and T. Shen *et al*., 2013. Mycology, cultivation, traditional uses, phytochemistry and pharmacology of *Wolfiporia cocos* (Schwein.) Ryvarden et Gilb.: A review. J. Ethnopharmacol., 147: 265-276.
2. Chu, B.F., H.C. Lin, X.W. Huang, H.Y. Huang, C.P. Wu and M.C. Kao, 2016. An ethanol extract of *Poria cocos* inhibits the proliferation of non-small cell lung cancer A549 cells via the mitochondria-mediated caspase activation pathway. J. Funct. Foods, 23: 614-627.
3. Kaminaga, T., K. Yasukawa, H. Kanno, T. Tai, Y. Nunoura and M. Takido, 1996. Inhibitory effects of lanostane-type triterpene acids, the components of *Poria cocos*, on tumor promotion by 12-O-tetradecanoylphorbol-13-acetate in two-stage carcinogenesis in mouse skin. Oncology, 53: 382-385.

4. Chen, X., L. Zhang and P.C.K. Cheung, 2010. Immunopotentiation and anti-tumor activity of carboxymethylated-sulfated β-(1→3)-D-glucan from Poria cocos. Int. Immunopharmacol., 10: 398-405.
5. Akihisa, T., Y. Nakamura, H. Tokuda, E. Uchiyama and T. Suzuki *et al*., 2007. Triterpene acids from *Poria cocos* and their anti-tumor-promoting effects. J. Nat. Prod., 70: 948-953.
6. Tang, J., J. Nie, D. Li, W. Zhu and S. Zhang *et al*., 2014. Characterization and antioxidant activities of degraded polysaccharides from *Poria cocos* sclerotium. Carbohydr. Polym., 105: 121-126.
7. Zhang, L., A.S. Ravipati, S.R. Koyyalamudi, S.C. Jeong and N. Reddy *et al*., 2013. Anti-fungal and anti-bacterial activities of ethanol extracts of selected traditional Chinese medicinal herbs. Asian Pac. J. Trop. Med., 6: 673-681.
8. Li, T.H., C.C. Hou, C.L. Chang and W.C. Yang, 2011. Anti-hyperglycemic properties of crude extract and triterpenes from *Poria cocos*. Evidence-Based Complement. Altern. Med., Vol. 2011. 10.1155/2011/128402.
9. Huang, Y.C., W.L. Chang, S.F. Huang, C.Y. Lin, H.C. Lin and T.C. Chang, 2010. Pachymic acid stimulates glucose uptake through enhanced GLUT4 expression and translocation. Eur. J. Pharmacol., 648: 39-49.
10. Cuellar, M.J., R.M. Giner, M.D.C. Recio, M.J. Just, S. Manez and J.L. Rios, 1997. Effect of the basidiomycete *Poria cocos* on experimental dermatitis and other inflammatory conditions. Chem. Pharmaceut. Bull., 45: 492-494.
11. Jeong, J.W., H.H. Lee, M.H. Han, G.Y. Kim, S.H. Hong, C. Park and Y.H. Choi, 2014. Ethanol extract of *Poria cocos* reduces the production of inflammatory mediators by suppressing the NF-kappaB signaling pathway in lipopolysaccharide-stimulated RAW 264.7 macrophages. BMC Complement. Altern. Med., Vol. 14. 10.1186/1472-6882-14-101
12. Cheng, S., I. Eliaz, J. Lin and D. Sliva, 2013. Triterpenes from *Poria cocos* suppress growth and invasiveness of pancreatic cancer cells through the downregulation of MMP-7. Int. J. Oncol., 42: 1869-1874.
13. Jin, Y., L. Zhang, M. Zhang, L. Chen, P.C.K. Cheung, V.E.C. Oi and Y. Lin, 2003. Antitumor activities of heteropolysaccharides of *Poria cocos* mycelia from different strains and culture media. Carbohydr. Res., 338: 1517-1521.
14. Kikuchi, T., E. Uchiyama, M. Ukiya, K. Tabata, Y. Kimura, T. Suzuki and T. Akihisa, 2011. Cytotoxic and apoptosis-inducing activities of triterpene acids from *Poria cocos*. J. Nat. Prod., 74: 137-144.
15. Li, X., Y. He, P. Zeng, Y. Liu and M. Zhang *et al*., 2019. Molecular basis for *Poria cocos* mushroom polysaccharide used as an antitumour drug in China. J. Cell. Mol. Med., 23: 4-20.
16. Gupta, M. and V. Mysore, 2016. Classifications of patterned hair loss: A review. J. Cutaneous Aesth. Surg., 9: 3-12.
17. Vary, Jr. J.C., 2015. Selected disorders of skin appendages-acne, alopecia, hyperhidrosis. Med. Clin. North Am., 99: 1195-1211.
18. Alonso, L. and E. Fuchs, 2006. The hair cycle. J Cell Sci., 119: 391-393.
19. Blanpain, C., W.E. Lowry, A. Geoghegan, L. Polak and E. Fuchs, 2004. Self-renewal, multipotency and the existence of two cell populations within an epithelial stem cell niche. Cell, 118: 635-648.
20. Tsai, F.M., C.H. Li, L.K. Wang, M.L. Chen, M.C. Lee, Y.Y. Lin and C.H. Wang, 2019. Extracellular signal-regulated kinase mediates ebastine-induced human follicle dermal papilla cell proliferation. BioMed Res. Int., Vol. 2019. 10.1155/2019/6360503.
21. Zhu, M. and R.C. Li, 1999. Receptor binding activities of *Schefflera triterpenoids* and oligosaccharides. Planta Med., 65: 99-103.
22. Park, Y.H., I.H. Son, B. Kim, Y.S. Lyu, H.I. Moon and H.W. Kang, 2009. *Poria cocos* Water Extract (PCW) protects PC12 neuronal cells from beta-amyloid-induced cell death through antioxidant and antiapoptotic functions. Die Pharmazie: Int. J. Pharmaceut. Sci., 64: 760-764.
23. Lee, D., S. Lee, S.H. Shim, H.J. Lee and Y. Choi *et al*., 2017. Protective effect of lanostane triterpenoids from the sclerotia of *Poria cocos* Wolf against cisplatin-induced apoptosis in LLC-PK1 cells. Bioorg. Med. Chem. Lett., 27: 2881-2885.
24. Azzouni, F., A. Godoy, Y. Li and J. Mohler, 2012. The 5 alpha-reductase isozyme family: A review of basic biology and their role in human diseases. Adv. Urol., Vol. 2012. 10.1155/2012/530121.
25. Rastegar, H., H.A. Ashtiani, M. Aghaei, B. Barikbin and A. Ehsani, 2015. Herbal extracts induce dermal papilla cell proliferation of human hair follicles. Ann. Dermatol., 27: 667-675.
26. Wang, T., 2003. The effects of the potassium channel opener minoxidil on renal electrolytes transport in the loop of Henle. J. Pharmacol. Exp. Therapeut., 304: 833-840.
27. Goren, A., J.A. Castano, J. McCoy, F. Bermudez and T. Lotti, 2014. Novel enzymatic assay predicts minoxidil response in the treatment of androgenetic alopecia. Dermatol. Ther., 27: 171-173.
28. Roberts, J., N. Desai, J. McCoy and A. Goren, 2014. Sulfotransferase activity in plucked hair follicles predicts response to topical minoxidil in the treatment of female androgenetic alopecia. Dermatol. Ther., 27: 252-254.
29. Ohyama, M., A. Shimizu, K. Tanaka and M. Amagai, 2010. Experimental evaluation of ebastine, a second-generation anti-histamine, as a supportive medication for alopecia areata. J. Dermatol. Sci., 58: 154-157.