Porcine Placenta Extract Enhances Ceramide Synthase 3 Expression through the PPARδ/ILK/Akt/mTOR/STAT3 Pathway

Akihiro Aioi[1,2*], Ryuta Muromoto[2], Jun-ichi Kashiwakura[2], Sho Mogami[1], Megumi Nishikawa[1], Shigeyuki Ogawa[1], Tadashi Matsuda[2]

[1]Septem-Soken 2-4-27 Dojima Kita-ku, Osaka, Japan
[2]Laboratory of Immunology, Faculty of Pharmaceutical Sciences, Hokkaido University, Sapporo, Japan
Email: *a-aioi@septem-so.com

Abstract

Porcine placenta extract (PPE) is widely accepted as an ingredient in complementary and alternative medicine and previous studies have reported its availability, however, its underlying action mechanism remains unclear. In this study, we investigated the underlying mechanism of PPE-induced ceramide synthase 3 upregulation. PPE enhanced the expression of ceramide synthase 3 at both the mRNA and protein levels in HaCaT cells. Moreover, PPE-induced ceramide synthase3 upregulation was suppressed by the Akt inhibitor, suggesting the involvement of Akt in the underlying mechanism. As the PI3K inhibitor did not affect PPE-induced ceramide synthase 3 upregulation, the factors upstream of Akt were estimated. Inhibition and small interfering RNA experiments demonstrated that the peroxisome proliferator-activated receptors δ (PPARδ) and integrin-linked kinase (ILK) are involved in the phosphorylation of Akt. Next, we explored the factors downstream of Akt and found that PPE induced phosphorylation of the STAT3 while PPE-induced upregulation of ceramide synthase 3 was significantly suppressed by the inhibitor of the mTOR, suggesting that the mTOR/STAT3 pathway is involved in the downstream of Akt. These results demonstrate that PPE upregulates CerS3 expression via the PPARδ/ILK/Akt/mTOR/STAT3 pathway.

Keywords

1. Introduction

The primary function of the epidermis is to construct an indispensable barrier between the organism and the desiccated terrestrial environment preventing invasion of external stimuli and loss of water from the body. As summarized in a previous study, many factors are involved in epidermal barrier function [1] [2]. Lipids, which are stored in lamellar bodies in the stratum granulosum and secreted into the extracellular space, are crucial for epidermal barrier function [3]. Previous studies have reported that lipid synthesis is regulated by various inter- and intracellular signaling pathways. Epidermal barrier disruption induces acute upregulation of the expression of inflammatory cytokines in the epidermis, although a chronic increase in cytokine production could have a harmful effect leading to inflammation and epidermal hyperproliferation [4]. Previous studies have suggested that inflammatory cytokines such as interleukin (IL)-6 and tumor necrosis factor (TNF) are potent stimulators of lipid synthesis to maintain epidermal homeostasis [5] [6]. In contrast, previous studies have demonstrated that peroxisome proliferator-activated receptor (PPAR) activators not only significantly enhance the synthesis of lipids including barrier-specific ceramide species, but also significantly accelerate the lamellar body formation, secretion, and post-secretory processing [7]. In addition, Mizutani *et al.* reported that activators of PPARβ/δ and PPARγ induce ceramide synthase 3 (CerS3) and that PPARβ/δ small interfering RNA (siRNA) inhibits the induction of CerS3 mRNA during differentiation [8]. These results suggest that PPARs play roles in the maintenance of epidermal barrier homeostasis.

Clinical applications of human and/or porcine placenta extract (PPE) are widely accepted in oriental medicine because the placenta is a rich reservoir of diverse bioactive molecules. A previous study showed that injection of human placenta extract into wound boundaries accelerated the decrease in wound size [9]. Moreover, oral administration of PPE reportedly reduces UVB-induced wrinkle formation in mice, along with the downregulation of matrix metalloproteinase (MMP)-2 mRNA expression [10]. In addition, studies using skin cells have revealed that human placenta extract accelerates keratinocyte and fibroblast proliferation [11] [12]. Recently, we reported that the application of PPE-containing gel on the face alleviated wrinkle formation and improved skin hydration, along with the upregulation of CerS3 in keratinocytes [13]. However, as the underlying mechanism still remains unclear, we explored the signaling pathway of PPE-induced CerS3 induction in this study.

2. Materials and Methods

2.1. Preparation of PPE

PPE was kindly provided by Kasyu Industries (Kitakyushu, Fukuoka, Japan). Briefly, fresh porcine placenta, collected at calving at the farm, was washed with water and then subjected to the freeze (−20˚C)-thawing (under 10˚C) cycle. After pasteurization at 65˚C for 6 h and removal of debris with 0.22 μm filter, the

obtained freeze-thaw drip was diluted with sterilized water to a nitrogen content of 0.27% to 0.33%, according to the Kjeldahl method described in the Japanese Standards of Food Additive.

2.2. Reagents and Antibodies

All inhibitors and an activator were purchased from Selleck Chemicals (Houston, TX, USA). Primary antibodies against β-actin antibody, CerS3, p-Akt (Ser473) and p-STAT3 were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA), Thermo Fisher Scientific (Waltham, MA, USA), and Cell Signaling Technology (Beverly, MA, USA), respectively.

2.3. Cells

To maintain the differentiation stage of HaCaT cells, calcium in fetal bovine serum was depleted by incubation with Chelex 100 resin (BioRad, Hercules, CA, USA) for 1 h at 4°C. HaCaT cells were maintained in Ca^{2+}-free Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% Ca^{2+}-depleted fetal bovine serum, 4 mM glutamine, 1 mM sodium pyruvate and 2 mM $CaCl_2$ at 37°C in a 5% carbon dioxide (CO_2-)-humidified atmosphere.

2.4. Treatment with PPE and Reagents

HaCaT cells were seeded into 24-well plates at a cell density of 1×10^5 cells/well for qPCR and into 6-well plates at a cell density of 3×10^5 cells/well for immunoblotting, and then maintained in a 5% CO_2-humidified atmosphere at 37°C. After cultivation for 24 h, the cells were treated with 5% PPE in the presence or absence of reagents for appropriate periods.

2.5. siRNA Transfection

HaCaT cells were reverse-transfected with predesigned PPARδ, and ILK siRNA (Thermo Fisher Scientific, Waltham, MA), according to the manufacturer's instructions. Cells were seeded into 24-well plates at a cell density of 1×10^5 cells/well for qPCR and into 6-well plates at a cell density of 3×10^5 cells/well for immunoblotting, and incubated in a humidified atmosphere of 5% CO_2 at 37°C for 24 h, followed by treatment with PPE and reagents for appropriate periods. The harvested cells were subjected to qPCR and immunoblotting assay.

2.6. RNA Isolation and qPCR

After the treatments, cells were harvested and total RNA was prepared with SV RNA isolation kit (Promega, Madison, WI, USA), according to the manufacturer's instructions, followed by reverse transcription using ReverTra Ace® qPCR RT Master Mix (TOYOBO, Osaka, Japan). PCR amplification and detection were conducted on a CFX96 real-time PCR system (BioRad, Hercules, CA, USA) using a KAPA SYBR FAST qPCR master mix (KAPA Biosystems, Woburn, MA, USA). The following primer pairs were used: β-actin,

5'-GATGAGATTGGCATGGCTTT-3' (sense) and
5'-CACCTTCACCGTTCCAGTTT-3' (antisense); CerS3,
5'-ACATTCCACAAGGCAACCATTG-3' (sense) and
5'-CTCTTGATTCCGCCGACTCC-3' (antisense); PPARδ,
5'-ACTGAGTTCGCCAAGAGCAT-3' (sense) and
5'-TGCACGCCATACTTGAGAAG-3' (antisense); ILK,
5'-AAGGTGCTGAAGGTTCGAGA-3' (sense) and
5'-ATACGGCATCCAGTGTGTGA-3' (antisense). The expression of target mRNA was quantified using the comparative threshold cycle (Ct) method for relative quantification ($2^{-\delta\delta Ct}$), normalized to the geometric mean of the reference gene β-actin.

2.7. Immunoblotting

HaCaT cells were collected in the RIPA buffer supplemented with protease inhibitors and phosphatase inhibitors. Equal amount of protein (10 µg) were loaded, resolved via SDS-PAGE and transferred to PVDF membrane, followed by immunoblotting with each antibody. Immunoreactive proteins were visualized using an enhanced chemiluminescence detection system (Millipore, Bedford, MA, USA). The relative intensity of the blot was measured using the ImageJ software and was normalized by the intensity of β-actin blot.

2.8. Statistical Analysis

Results of mRNA relative expression and densitometric analysis of western blotting images are expressed as the mean ± standard deviation (SD) of at least three independent experiments. Statistical analysis was performed using the Student's t-test. Statistical significance was set $p < 0.05$.

3. Results

3.1. PPE Enhanced CerS3 Expression

To confirm the effect of PPE on the mRNA expression of CerS3, quantitative polymerase chain reaction (qPCR) was performed. The treatment with PPE significantly upregulated the mRNA expression of CerS3 to 2.40 ± 0.59 (Figure 1(a)). As well, the protein expression of CerS3 was significantly enhanced to 2.00 ± 0.58 (Figure 1(b) and Figure 1(c)).

3.2. Akt Was Involved in PPE-Induced Upregulation of CerS3

GSK690693, an inhibitor of Akt, was employed to evaluate the involvement of Akt in PPE-enhanced CerS3 mRNA and protein expression. Treatment with GSK690693 significantly downregulated PPE-enhanced CerS3 mRNA expression (Figure 2(a)). In addition, the protein level of CerS3 was significantly decreased by treatment with GSK690693 (Figure 2(b) and Figure 2(c)). In contrast, LY294002, an inhibitor of phosphoinositide 3-kinase (PI3K) that phosphorylates Akt, did not affect PPE-induced upregulation of CerS3 expression (Figure 2(d)).
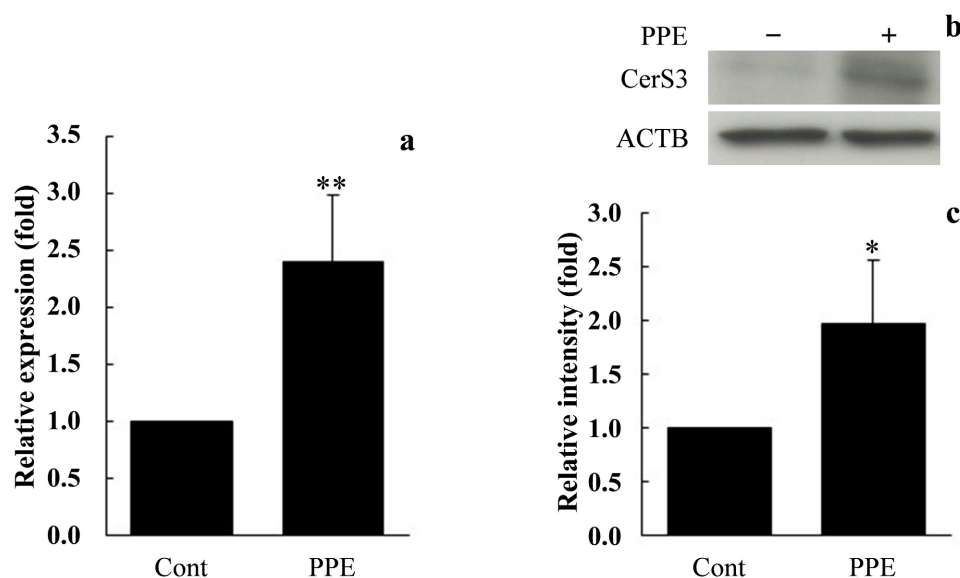
Figure 1. Porcine placenta extract (PPE) enhanced ceramide synthase 3 (CerS3) expression. (a) CerS3 mRNA expression was upregulated in PPE-treated cells. (b) Representative western blotting image of equal amounts of protein extracts from the control and PPE-treated cells. (c) Relative intensity of CerS3 blots was measured using the ImageJ software and was normalized by the intensity of the β-actin blot. Statistical analyses were performed with a Student's t-test. *p-value < 0.05, **p-value < 0.01 were considered to be significantly different from the control.

3.3. PPARδ Was Involved in PPE-Induced Upregulation of CerS3

The PPARδ selective inhibitor, GSK3787, significantly downregulated PPE-enhanced CerS3 mRNA expression, whereas GSK9662, a selective inhibitor of PPARγ, did not affect PPE-enhanced CerS3 mRNA expression (**Figure 3(a)**). Moreover, GW0742, a PPARδ activator, upregulated CerS3 mRNA expression as well as PPE (**Figure 3(b)**). While the expression of PPARδ was knocked down by siRNA (**Figure 3(c)**), the effect of PPE on CerS3 expression was diminished (**Figure 3(d)**). In addition, GSK3787 and PPARδ knockdown (KD) significantly reduced the upregulation of CerS3 protein levels by PPE treatment (**Figures 3(e)-(h)**).

3.4. Integrin-Kinked Kinase (ILK) Was Involved in PPE-Induced Upregulation of CerS3

The ILK inhibitor, OSU-T315, drastically reduced the effect of PPE on CerS3 expression at both the mRNA and protein levels (**Figures 4(a)-(c)**). While the expression of ILK was knocked down by siRNA (**Figure 4(d)**), the upregulation of CerS3 expression by PPE was diminished significantly at both the mRNA and protein levels (**Figures 4(e)-(g)**). The mRNA expression of ILK was significantly enhanced after 16 h of PPE treatment, following significant upregulation of PPARδ mRNA expression (**Figure 4(h)**).

3.5. mTOR/STAT3 Signaling Pathway Participated in PPE-Induced Upregulation of CerS3

The PPE-induced CerS3 expression at the mRNA and protein levels was signifi-

cantly reduced in the presence of rapamycin, an mTOR inhibitor (**Figures 5(a)-(c)**). Phosphorylated STAT3 (p-STAT3) was detected in HaCaT cells treated with PPE, as well as phosphorylated Akt (**Figure 5(d)**), suggesting the involvement of STAT3 in PPE-induced CerS3 upregulation.
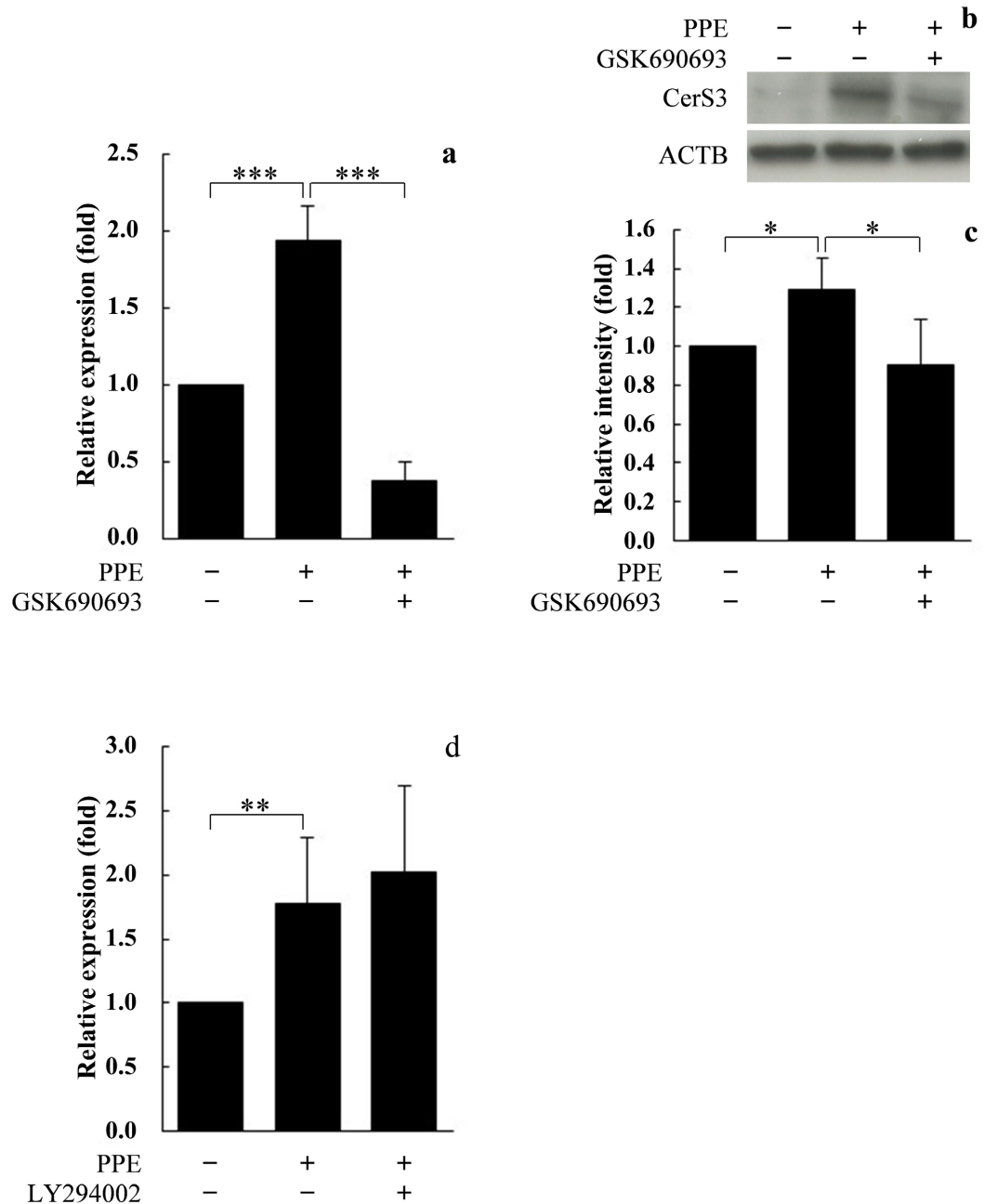

Figure 2. Akt was involved in PPE-induced upregulation of CerS3 expression, but not PI3K. (a) GSK690693 suppressed CerS3 mRNA expression upregulated by PPE treatment. (b) Representative western blotting image of equal amounts of protein extracts from the control and PPE-treated cells in the absence and presence of GSK690693. (c) Relative intensity of CerS3 blots was measured using the ImageJ software and was normalized by the intensity of the β-actin blot. (d) LY294002 did not affect PPE-upregulated CerS3 mRNA expression. Statistical analyses were performed with a Student's t-test. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001 were considered to be significantly different from the control.
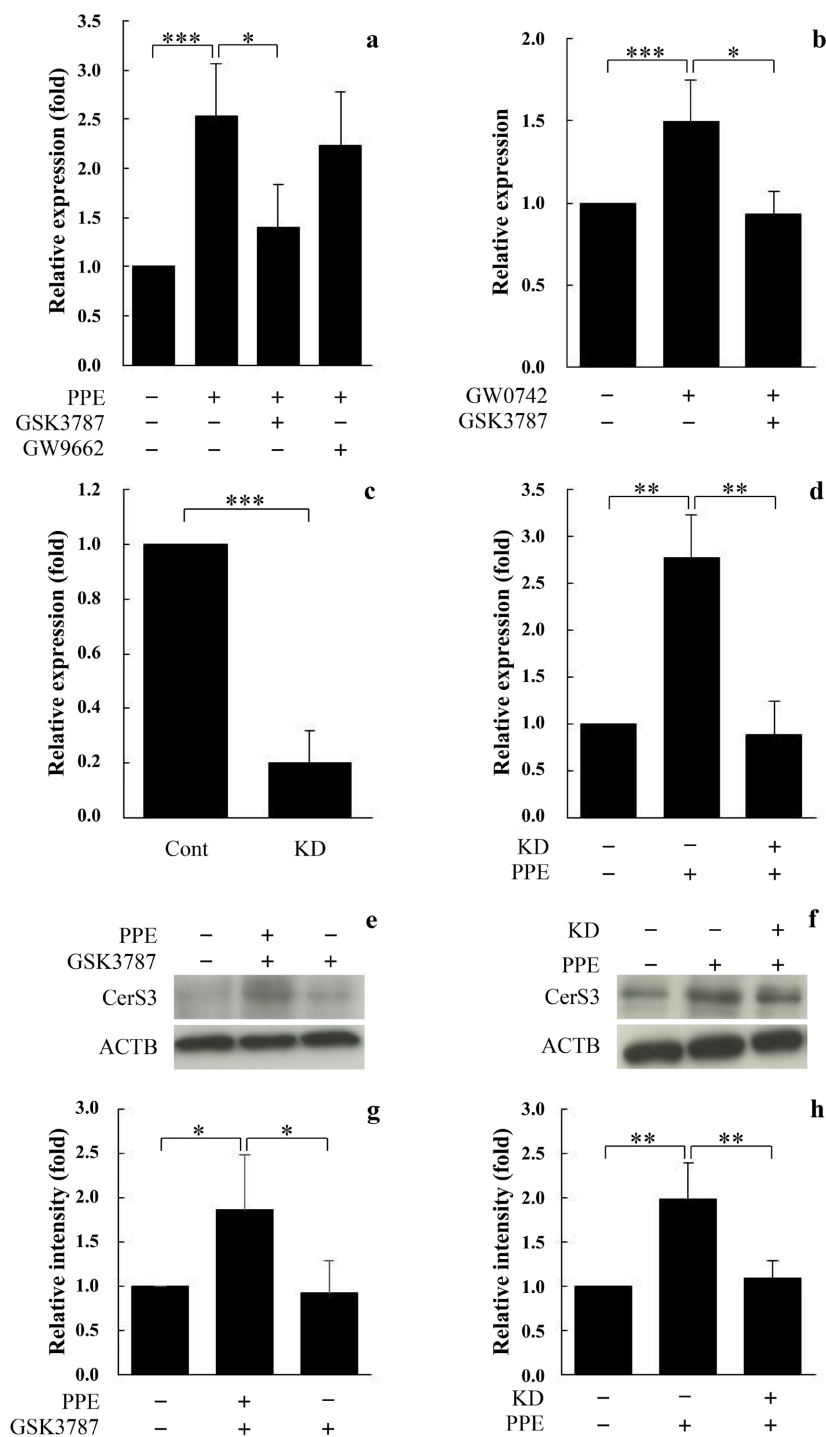
Figure 3. PPARδ was involved in PPE-induced upregulation of CerS3 expression, but not PPARγ. (a) GSK3787 suppressed CerS3 mRNA expression upregulated by PPE treatment, while GW9662 did not affect the upregulation. (b) GW0742 enhanced CerS3 mRNA expression and its upregulation was suppressed by GSK3787. (c) Expression of PPARδ mRNA was reduced by the small interfering RNA (siRNA). (d) PPARδ knockdown (KD) suppressed CerS3 mRNA expression upregulated by PPE treatment. (e) Representative western blotting image of equal amounts of protein extracts from the control and PPE-treated cells in the absence and presence of GSK3787. (f) Representative western blotting image of equal amounts of protein extracts from the control and PPARδ KD cells. (g and h) Relative intensity of CerS3 blots was measured using the ImageJ software and was normalized by the intensity of the β-actin blot in inhibition and siRNA experiments. Statistical analyses were performed with a Student's t-test. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001 were considered to be significantly different from the control.
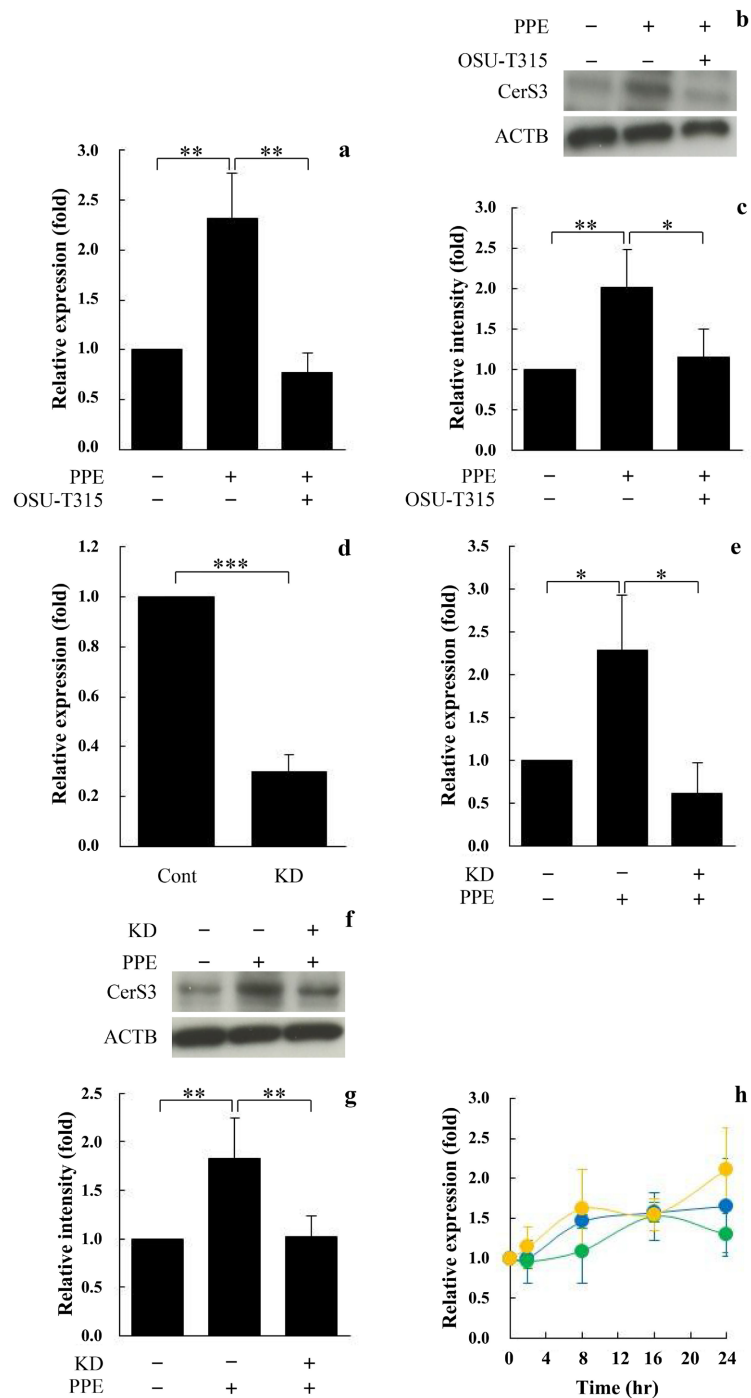
Figure 4. ILK was involved in PPE-induced upregulation of CerS3 expression. (a) OSU-T315 suppressed CerS3 mRNA expression upregulated by PPE treatment. (b) Representative western blotting image of equal amounts of protein extracts from the control and PPE-treated cells in the absence and presence of OSU-T315. (c) Relative intensity of CerS3 blots was measured using the ImageJ software and was normalized by the intensity of the β-actin blot in the inhibition experiments. (d) Expression of ILK mRNA was reduced by siRNA. (e) ILK KD suppressed CerS3 mRNA expression upregulated by PPE treatment. (f) Representative western blotting image of equal amounts of protein extracts from the control and the ILK KD cells. (g) Relative intensity of CerS3 blots was measured using the ImageJ software and was normalized by the intensity of the β-actin blot in the siRNA experiments. (h) CerS3 mRNA expression (yellow circle) arose in the acute phase (after 2 hr) and was maintained throughout the experimental period. PPARδ mRNA expression (blue circle) arose after 8 hr, followed by ILK mRNA expression (green circle). Statistical analyses were performed with a Student's t-test. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001 were considered to be significantly different from the control.
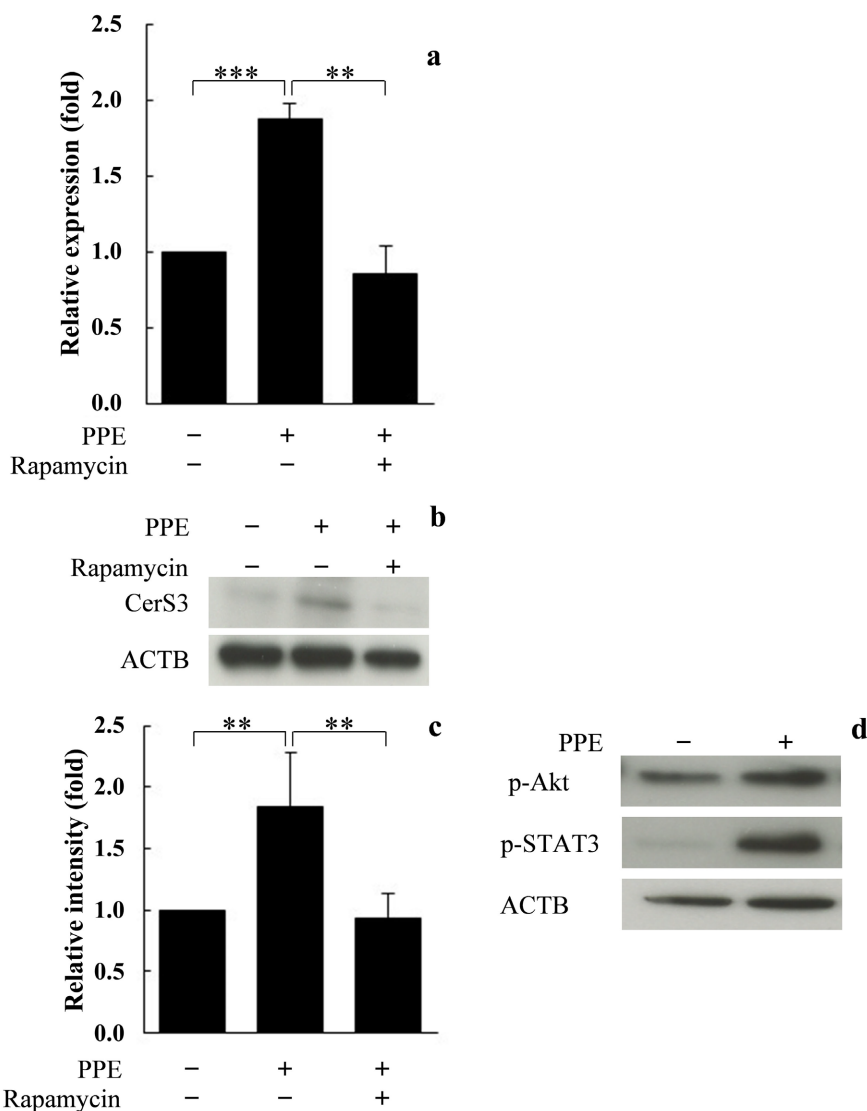
Figure 5. mTOR)/STAT3 signaling pathway was involved in PPE-induced upregulation of CerS3 expression. (a) Rapamycin, an mTOR inhibitor, suppressed CerS3 mRNA expression upregulated by PPE treatment. (b) Representative western blotting image of equal amounts of protein extracts from the control and PPE-treated cells in the absence and presence of rapamycin. (c) Relative intensity of CerS3 blots was measured using the ImageJ software and was normalized by the intensity of the β-actin blot in inhibition experiments. (d) Representative western blotting image of p-Akt and p-STAT3 in the cells treated with PPE for 30 min. Statistical analyses were performed with a Student's t-test. *p-value < 0.05, **p-value < 0.01 and ***p-value < 0.001 were considered to be significantly different from the control.

4. Discussion

Ceramides, which are the predominant lipids comprising 30% to 50% of the intercellular lipid content in the stratum corneum, are synthesized by the enzymatic actions of ceramide synthases (CerS). CerS3, which is one of the six distinct isoforms of CerSs, is highly expressed in keratinocytes, synthesizes ceramides containing α-hydroxy fatty acids, and is involved in maintaining skin bar-

rier function [14] [15] [16]. We have recently reported that PPE, which is widely used in alternative and traditional medicine, enhances CerS3 expression [13]. However, because the regulation of CerS3 expression is largely uncharacterized, we explored the underlying mechanism of the PPE-induced upregulation of CerS3 in this study. The expression levels of CerS3 mRNA and protein were significantly upregulated in the cells treated with PPE (Figure 1). The upregulation was diminished by Akt inhibitor (Figure 2(a) and Figure 2(b)), although LY294002, a PI3K inhibitor, did not affect the effect of PPE (Figure 2(c)). This suggests that some signaling pathways other than PI3K/Akt are involved in the mechanism underlying the induction of CerS3. To elucidate the upstream and downstream pathways of Akt, we first examined the involvement of PPARδ in the signaling pathway. As shown in Figure 3, PPARδ is involved in the PPE-induced upregulation of CerS3 expression. This corresponds to a previous study demonstrating that PPARβ/δ is involved in CerS3 upregulation during keratinocyte differentiation [8]. However, to the best of our knowledge, the binding site of PPARδ does not exist in the promoter region of the *CerS3* gene, suggesting that PPARδ contributes indirectly to CerS3 upregulation. As described above, Akt is crucial in the signaling pathway involved in PPE-induced CerS3 upregulation, as well as PPARδ. A previous study demonstrated that ILK, which is ubiquitously expressed, phosphorylates Akt at serine 473, leading to the activation of Akt [17] [18]. The inhibition of ILK and ILK KD diminished PPE-induced CerS3 upregulation (Figures 4(a)-(g)), suggesting that ILK also plays a pivotal role in the signaling pathway of the effect of PPE. Di-Poï *et al.* reported that PPARβ/δ modulates Akt activation via the upregulation of ILK [19]. As shown in Figure 4(h), PPE treatment enhanced PPARδ mRNA expression, followed by the upregulation of ILK mRNA expression, suggesting that enhancement of the expression of PPARδ and ILK contributes to maintaining the upregulated level of CerS3 expression during the experimental period. Collectively, the PPE treatment regulates Akt phosphorylation via ILK whose expression is enhanced by PPARδ upstream of the signaling pathway of PPE-induced CerS3 upregulation. An important effector of Akt via tuberous sclerosis (TSC)-1/2 and the small GTPase Rheb is the mTOR signaling pathway [20] [21]. Therefore, we elucidated the involvement of mTOR in the signaling pathway involved in PPE-induced CerS3 upregulation. Rapamycin, an mTOR inhibitor, suppressed PPE-upregulated CerS3 expression at both the mRNA and protein levels (Figures 5(a)-(c)), suggesting the involvement of mTOR in the signaling pathway. Next, the question arose as to what transcription factors are located downstream of the mTOR signaling pathway. Previous studies demonstrated that mTOR activates various transcription factors such as PPARα and HIF-1α [22] [23]. In addition, a previous study showed that mTOR also positively regulates STAT3 activation in in breast cancer stem-like cells [24]. STAT3 belongs to a family of cytoplasmic protein. Once activated by phosphorylation, STAT3 forms homodimers, enters into the nucleus and initiates the expression of target genes. Because STAT3 was

identified as a component of the interleukin-6-activated acute phase response factor complex, its role in innate immunity has been actively studied [25]. Additionally, subsequent studies have revealed the role of STAT3 in various cell types [26] [27]. In keratinocytes, STAT3 plays a crucial role in keratinocyte migration and skin remodeling [28]. Therefore, we examined the activation of STAT3 in PPE-treated cells. As expected, phosphorylated STAT3 was detected in PPE-treated cells (**Figure 5(d)**). Collectively, the mTOR/STAT3 signaling pathway participates downstream of Akt phosphorylation in PPE-upregulated CerS3 expression. In conclusion, we found that PPE upregulated CerS3 expression via the PPARδ/ILK/Akt/mTOR/STAT3 signaling pathway (**Figure 6**). In particular, PPE induced the persistent expression of PPARδ, while ILK contributed to the enhanced expression of CerS3.
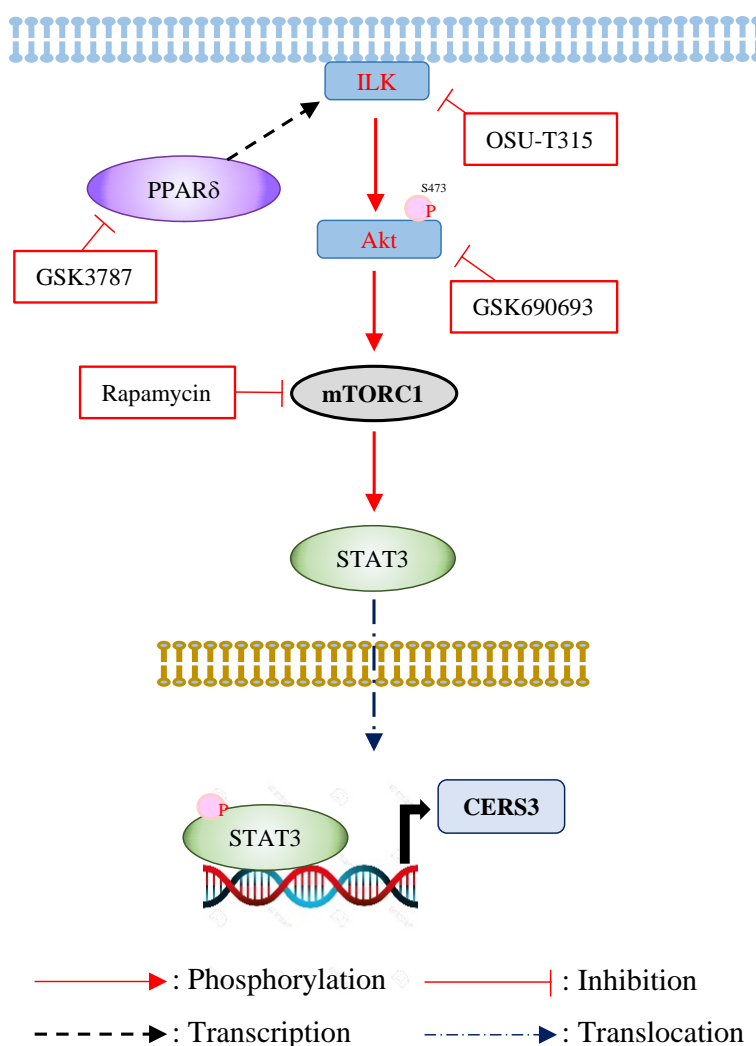

Figure 6. Potential signaling pathway underlying the PPE-induced upregulation of CerS3.

5. Conclusion

In this study, we demonstrated, for the first time, the underlying mechanism of

PPE function and provided scientific evidence for the application of PPE in alternative medicine. Combined with our previous study showing that applying a gel containing placental extract to humans increases the water content in the stratum corneum [13], this study suggests that placental extract improves skin barrier function by increasing ceramide contents via the upregulation of CerS3 expression level. However, further research to show that placental extract increases ceramide in human skin in vivo is required.

Availability of Data and Materials

All data generated or analyzed during this study are included in this published article.

Acknowledgements

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

[1] Natsuga, K. (2014) Epidermal Barriers. *Cold Spring Harbor Perspective in Medicine*, **4**, a018218. https://doi.org/10.1101/cshperspect.a018218

[2] Proksch, E., Brandner, J.M. and Jensen, J.M. (2008) The Skin: An Indispensable Barrier. *Experimental Dermatology*, **12**, 1063-1072. https://doi.org/10.1111/j.1600-0625.2008.00786.x

[3] Madison, K.C. (2003) Barrier Function of the Skin: "la raison d'être" of the Epidermis. *Journal of Investigative Dermatology*, **121**, 231-241. https://doi.org/10.1046/j.1523-1747.2003.12359.x

[4] Wood, L.C., Jackson, S.M., Elias, P.M., Grunfeld, C. and Feingold, K.R. (1992) Cutaneous Barrier Perturbation Stimulates Cytokine Production in the Epidermis of Mice. *Journal of Clinical Investigation*, **90**, 482-487. https://doi.org/10.1172/JCI115884

[5] Wang, X.P., Schunck, M., Kallen, K.J., Neumann, C., Trautwein, C., Rose-John, S. and Proksch, E. (2004) The Interleukin-6 Cytokine System Regulates Epidermal Permeability Barrier Homeostasis. *Journal of Investigative Dermatology*, **123**, 124-131. https://doi.org/10.1111/j.0022-202X.2004.22736.x

[6] Jensen, J.M., Schütze, S., Förl, M., Krönke, M. and Proksch, E. (1999) Roles for Tumor Necrosis Factor Receptor p55 and Sphingomyelinase in Repairing the Cutaneous Permeability Barrier. *Journal of Clinical Investigation*, **104**, 1761-1770. https://doi.org/10.1172/JCI5307

[7] Man, M.Q., Choi, E.H., Schmuth, M., Crumrine, D., Uchida, Y., Elias, P.M., Holleran, W.M. and Feingold, K.R. (2006) Basis for Improved Permeability Barrier Homeostasis Induced by PPAR and LXR Activators: Liposensors Stimulate Lipid Synthesis, Lamellar Body Secretion, and Post-Secretory Lipid Processing. *Journal of Investigative Dermatology*, **126**, 386-392. https://doi.org/10.1038/sj.jid.5700046

[8] Mizutani, Y., Sun, H., Ohno, Y., Sassa, T., Wakashima, T., Obara, M., Yuyama, K.,

Kihara, A. and Igarashi, Y. (2013) Cooperative Synthesis of Ultra Long-Chain Fatty Acid and Ceramide during Keratinocyte Differentiation. *PLOS ONE*, **8**, e67317. https://doi.org/10.1371/journal.pone.0067317

[9] Hong, J.W., Lee, W.J., Hahn, S.B., Kim, B.J. and Lew, D.H. (2010) The Effect of Human Placenta Extract in a Wound Healing Model. *Annals of Plastic Surgery*, **65**, 96-100. https://doi.org/10.1097/SAP.0b013e3181b0bb67

[10] Hong, K.B., Park, Y., Kim, J.H., Kim, J.M. and Suh, H.J. (2015) Effects of Porcine Placenta Extract Ingestion on Ultraviolet B-Induced Skin Damage in Hairless Mice. *Korean Journal of Food Science of Animal Resources*, **35**, 413-420. https://doi.org/10.5851/kosfa.2015.35.3.413

[11] O'Keefe, E.J., Payne, R.E. and Russell, N. (1985) Keratinocyte Growth-Promoting Activity from Human Placenta. *Journal of Cellular Physiology*, **124**, 439-445. https://doi.org/10.1002/jcp.1041240312

[12] Cho, H.R., Ryou, J.H., Lee, J.W. and Lee, M.H. (2008) The Effects of Placental Extract on Fibroblast Proliferation. *Journal of Cosmetic Science*, **59**, 195-202.

[13] Aioi, A., Muromoto, R., Mogami, S., Nishikawa, M., Ogawa, S. and Matsuda, T. (2021) Porcine Placenta Extract Reduced Wrinkle Formation by Potentiating Epidermal Hydration. *Journal of Cosmetic Dermatological Science Application*, **11**, 101-109. https://doi.org/10.4236/jcdsa.2021.112011

[14] Sassa, T., Hirayama, T. and Kihara, A. (2016) Enzyme Activities of the Ceramide Synthases CERS2-6 Are Regulated by Phosphorylation in the C-Terminal Region. *Journal of Biological Chemistry*, **291**, 7477-7487. https://doi.org/10.1074/jbc.M115.695858

[15] Mullen, T.D., Hannun, Y.A. and Obeid, L.M. (2012) Ceramide Synthases at the Centre of Sphingolipid Metabolism and Biology. *Biochemistry Journal*, **441**, 789-802. https://doi.org/10.1042/BJ20111626

[16] Levy, M. and Futerman, A.H. (2010) Mammalian Ceramide Synthases. *IUBMB Life*, **62**, 347-356. https://doi.org/10.1002/iub.319

[17] Nicholson, K.M. and Anderson, N.G. (2002) The Protein Kinase B/Akt Signaling Pathway in Human Malignancy. *Cellular Signaling*, **14**, 381-395. https://doi.org/10.1016/S0898-6568(01)00271-6

[18] Persad, S., Attwell, S., Gray, V., Mawji, N., Deng, J.T., Leung, D., Yan, J., Sanghera, J., Walsh, M.P. and Dedhar, S. (2001) Regulation of Protein Kinase B/Akt-Serine 473 Phosphorylation by Integrin-Linked Kinase: Critical Roles for Kinase Activity and Amino Acids Arginine 211 and Serine 343. *Journal of Biological Chemistry*, **276**, 27462-27469. https://doi.org/10.1074/jbc.M102940200

[19] Di-Poï, N., Tan, N.S., Michalik, L., Wahli, W. and Desvergne, B. (2002) Antiapoptotic Role of PPARβ in Keratinocytes via Transcriptional Control of the Akt1 Signaling Pathway. *Molecular Cell*, **10**, 721-733. https://doi.org/10.1016/S1097-2765(02)00646-9

[20] Dibble, C.C. and Cantley, L.C. (2015) Regulation of mTORC1 by PI3K Signaling. *Trends in Cell Biology*, **25**, 545-555. https://doi.org/10.1016/j.tcb.2015.06.002

[21] Buerger, C., Shirsath, N., Lang, V., Berard, A., Diehl, S., Kaufmann, R., Boehncke, W.H. and Wolf, P. (2017) Inflammation Dependent mTORC1 Signaling Interferes with the Switch from Keratinocyte Proliferation to Differentiation. *PLOS ONE*, **12**, e0180853. https://doi.org/10.1371/journal.pone.0180853

[22] Li, J., Huang, Q., Long, X., Zhang, J., Huang, X., Aa, J., Yang, H., Chen, Z. and Xing, J. (2015) CD147 Reprograms Fatty Acid Metabolism in Hepatocellular Carcinoma Cells through Akt/mTOR/SREBP1c and P38/PPARa Pathways. *Journal of Hepatol-*

ogy, **63**, 1378-1389. https://doi.org/10.1016/j.jhep.2015.07.039

[23] Aioi, A. (2022) Royal Jelly Extract Accelerates Keratinocyte Proliferation, and Upregulates Laminin α3 and Integrin β1 mRNA Expression, via Akt/mTOR/HIF-1α Pathway. *Journal of Cosmetics, Dermatological Sciences and Applications*, **12**, 83-94. https://doi.org/10.4236/jcdsa.2022.122007

[24] Zhou, J., Wulfkuhle, J., Zhang, H., Gu, P., Yang, Y., Deng, J., Margolick, J.B., Liotta, L.A., Petricoin, E. and Zhang, Y. (2007) Activation of the PTEN/mTOR/STAT3 Pathway in Breast Cancer Stem-Like Cells Is Required for Viability and Maintenance. *Proceedings of the National Academy of Sciences USA*, **104**, 16158-16163. https://doi.org/10.1073/pnas.0702596104

[25] Hillmer, E.J., Zhang, H., Li, H.S. and Watowich, S.S. (2016) STAT3 Signaling in Immunity. *Cytokine Growth Factor Review*, **31**, 1-15. https://doi.org/10.1016/j.cytogfr.2016.05.001

[26] Bromberg, J. and Darnell, J.E. (2000) The Role of STATs in Transcriptional Control and Their Impact on Cellular Function. *Oncogene*, **19**, 2468-2473. https://doi.org/10.1038/sj.onc.1203476

[27] Levy, D.E. and Lee, C.K. (2002) What Does Stat3 Do? *Journal of Clinical Investigation*, **109**, 1143-1148. https://doi.org/10.1172/JCI0215650

[28] Sano, S., Itami, S., Takeda, K., Tarutani, M., Yamaguchi, Y., Miura, H., Yoshikawa, K., Akira, S. and Takeda, J. (1999) Keratinocyte-Specific Ablation of Stat3 Exhibits Impaired Skin Remodeling, But Does Not Affect Skin Morphogenesis. *EMBO Journal*, **18**, 4657-4668. https://doi.org/10.1093/emboj/18.17.4657

List of Abbreviations

Cers3: ceramide synthase 3; DMEM: Dulbecco's modified Eagle's medium; IL-6: interleukin-6; ILK: integrin-linked kinase; MMP-2: matrix metalloproteinase-2; mTOR: mammalian target of rapamycin; PI3K: phosphoinositide 3-kinase; PPAR: peroxisome proliferator-activated receptor; PPE: porcine placenta extract; PVDF: poly vinylidene difluoride; qPCR: quantitative polymerase chain reaction; RIPA: radio-immunoprecipitation assay; siRNA: small interfering RNA; SDS-PAGE: sodium dodecyl sulfate-polyacrylamide gel electrophoresis; STAT3: signal transducer and activator of transcription 3; TNF: tumor necrosis factor.