RESEARCH ARTICLE
doi: 10.2306/scienceasia1513-1874.2024.081

ScienceAsia 50 (4): 2024: ID 2024081: **1–9**

Taraxasterol protects osteoblasts from high glucose by regulating ERK 1/2

Yu Zhang[a,b], **Ramizu Bin Shaari**[a,*], **Mohamad Arif Bin Awang Nawi**[a], **Akram Bin Hassan**[a], **Caiyun Cui**[c]

[a] School of Dental Sciences, Universiti Sains Malaysia, Health Campus, Kelantan 16150 Malaysia
[b] Qilu Medical University, Zibo, Shandong 255213 China
[c] Department of Stomatology, Affiliated Hospital of Binzhou Medical University, Binzhou 256601 China

*Corresponding author, e-mail: ramizu@usm.my

Received 3 Feb 2024, Accepted 23 Jul 2024
Available online 19 Sep 2024

ABSTRACT: Chronic hyperglycemia in diabetic patients adversely affects osteoblast distribution, potentially leading to inflammation and cellular damage. Diabetic osteoporosis develops in part due to oxidative damage caused by high glucose (HG) levels. Taraxasterol has anti-inflammatory, anti-oxidative, and anti-tumor properties; it is derived from the Compositae plant Taraxacum. Preliminary research indicates taraxasterol potential to enhance osteoblast proliferation and differentiation, yet its protective properties in high-glucose conditions are not well-documented. We established a taraxasterol concentration of 10 μg/ml based on cell toxicity assays. Cell proliferation, alkaline phosphatase (ALP) activity, oxidative stress (OS) levels, and mRNA expression of osteogenic genes like Runt-related transcription factor 2 (Runx2) and ALP were measured in the study to determine the effect of taraxasterol on the MC3T3-E1 cell line in HG conditions. To assess the levels of phosphorylated ERK (p-ERK) and extracellular signal-regulated kinase (ERK), Western blot analyses were performed. The results showed taraxasterol highly expressed osteogenic markers such as ALP and Runx2 in the presence of HG, decreased OS, and accelerated the proliferation and differentiation of MC3T3-E1 under the HG condition. More importantly, it reduced the activation of the ERK pathway systematically activated by the presence of HG. Therefore, our study suggests that taraxasterol can mitigate OS and enhance the proliferation and differentiation of osteoblasts in HG environments. This effect may be associated with taraxasterol ability to inhibit the upregulation of ERK and p-ERK expression induced by HG.

KEYWORDS: taraxasterol, diabetes, high glucose, MC3T3-E1, oxidative stress

INTRODUCTION

Diabetes is a common disease in the world, leading to many diseases and complications [1]. HG levels have been ascertained to cause OS and inflammatory apoptosis, which subsequently cause inhibition in the proliferation, differentiation, and expression of osteogenic genes of the osteoblasts. An individual with diabetes is at higher risk of eventually developing osteoporosis [2, 3]. The MAPK family member ERK1/2 is involved in the transfer of extracellular signals to intracellular destinations [4]. HG levels promote phosphorylation of ERK and activation of the MAPK pathway in *in vitro* MC3T3-1 cells [5]. This could further limit osteoblast proliferation and differentiation by increasing OS [6]. Furthermore, stimulation of the ERK pathway may enhance osteoblast autophagy [7].

Taraxasterol is a kind of compound derived mainly from plants of the Compositae family, especially dandelion. This pentacyclic triterpenoid has the molecular formula as $C_{30}H_{50}O$ and molecular weight of 426.72 [8]. It is considered to show broad pharmacological effects [9]. Research has highlighted taraxasterol potential to mitigate inflammation, OS, and tumor formation. The suppression of the NF-κB and MAPK signaling pathways is thought to be the cause of its anti-inflammatory properties [10]. Meanwhile, taraxasterol also can promote the development and proliferation of osteoblasts, which contributes to the treatment of osteoporosis [11]. In the HG settings, taraxasterol antagonizes the pathway of ERK, which in turn activates typically inflammatory apoptosis in osteoblasts. Whether taraxasterol could protect osteoblasts from HG settings is fundamentally aimed at this study. Given that there are not too many choices that could effectively protect the bones of diabetic patients, the current study aims to assess the proper protective effects of taraxasterol on osteoblasts in an HG setting. The results will show that taraxasterol has a therapeutic effect on improving wellness, focusing, in particular, on bone strength in diabetes.

MATERIALS AND METHODS

Taraxasterol preparation

Prior to the experiment, taraxasterol (Meilunbio, China) was dissolved in dimethyl sulfoxide (DMSO; Solarbio, China), and the stock solution was stored at 4 °C. Various concentrations of taraxasterol (0 to 25 μg/ml) were prepared to conduct cell toxicity assays.

Cell cultures

The mouse embryo osteoblast cell line (MC3T3-E1), obtained from Dalian Meilun Biotechnology Co., Ltd., China, was cultured in α-MEM low-glucose medium

supplemented with 1% penicillin-streptomycin and 10% fetal bovine serum. Cells in logarithmic growth phase were used for experiments. Two culture conditions were established: a normal control (NC) group in α-MEM with 5.5 mmol/l glucose, and a high glucose (HG) group in α-MEM with 25.0 mmol/l glucose.

Detection of taraxasterol toxicity by CCK-8 assay

MC3T3-E1 cells were seeded at a density of 3×10^4 cells/ml with 100 µl per well in a 96-well plate. The experiment was divided into 6 groups based on different taraxasterol concentrations: 0, 5, 10, 15, 20, and 25 µg/ml. Each concentration group had 3 replicate wells, which were incubated at 37 °C with 5% CO_2 for 24 h. Then, each well received 100 µl of a mixture (CCK-8:culture medium = 1:9). After 2 h of incubation, absorbance at 450 nm was measured employing an ELISA reader to determine the viability of the cells. For further tests, the non-toxic concentration was chosen.

CCK-8 assay for cell proliferation

Three duplicate wells per condition were used to seed MC3T3-E1 cells at a density of 3×10^4 cells/ml into each well of a 96-well plate. To enable adhesion, the cells were cultivated for 24 h at 37 °C and 5% CO_2. The experiment included 4 groups: NC group, NC + taraxasterol group, HG group, and HG + taraxasterol group, with a taraxasterol concentration of 10 µg/ml. Cells were cultivated for 1, 4, and 7 days. Following each period, wells received 100 µl of a solution (CCK-8:culture media = 1:9) and were incubated for an additional 4 h. Absorbance was then measured at 450 nm using an ELISA reader.

Alkaline phosphatase activity assay

In a 6-well plate, MC3T3-E1 cells were seeded at a density of 3×10^4 cells/well and incubated at 37 °C with 5% CO_2 for 24 h to allow cell adherence. Then, the cells were divided into 4 groups: NC, NC + taraxasterol, HG, and HG + taraxasterol. A calcification induction solution was used to induce calcification, and the cells were cultured for 1, 4, and 7 days. The original medium was removed, and 200 µl of cell lysis buffer was added to each well. After sufficient lysis, the cells were collected and centrifuged to obtain the supernatant. As directed by the ALP kit (Beyotime Biotechnology Co., Ltd., China), an ELISA reader was used to measure the OD value at 405 nm.

Reactive oxygen species (ROS) detection

To prepare the Dichlorodihydrofluorescein diacetate (DCFH-DA, Servicebio, China) working solution, the DCFH-DA probe was diluted in serum-free cell culture medium at a 1:1000 ratio. MC3T3-E1 cells were seeded in a 6-well plate at a density of 3×10^4 cells/well and incubated at 37 °C with 5% CO_2 for 24 h to allow cell adherence. The cells were divided into groups as previously described. After another 24-h incubation, the cell culture medium was replaced with 2 ml of DCFH-DA working solution per well. After 2 min of incubation at 37 °C, the cells were washed 3 times in medium devoid of serum. The cells were observed using a fluorescence microscope.

Rhodamine-Phalloidin staining

The experimental groups remained unchanged. In laser confocal dishes, 3×10^4 cells/ml were used for seeding, and each well held 3 duplicates and 1 ml of cell suspension. Following a 24-h incubation period at 37 °C, the cells were fixed, and the culture medium was withdrawn. A 200 µl solution of Rhodamine-Phalloidin (100 nM, Servicebio) was added to cover the cells on the slides, followed by a 30-min incubation in the dark at room temperature. The cells were then stained with DAPI for the nucleus and washed 3 times with PBS. The cell skeleton morphology was analyzed using a laser scanning confocal microscope after applying an anti-fade reagent.

RT-qPCR analysis

The experimental groups were kept up as already explained. Then, 3×10^4 cells/ml of MC3T3-E1 medium were planted in each of the 6-well plates with 3 duplicates for each condition. After 24 h, the culture medium was refreshed according to the group assignments, and cells underwent osteogenic induction for 7 days. Subsequently, 1 ml of Trizol was added to each well, replacing the culture medium, to lyse the cells. Chloroform (0.2 ml) was added to each ml of Trizol, well mixed for 15 s, and allowed to remain at a room temperature for 5 min. After that, the mixture was centrifuged for 10 min at 12,000 rpm and 4 °C. The precipitate was washed and dissolved. RNA concentration was spectrophotometrically determined. RT-qPCR was used to assess the expression levels of Runx2 and ALP. Table S1 contains a list of primers used in this experiment.

Western blot analysis

MC3T3-E1 cells were lysed using RIPA buffer containing PMSF with the lysate kept on ice for 30 min and repeatedly pipetted to ensure complete cell lysis. The total protein was extracted from the supernatant after centrifuging the lysate at 12,000 rpm for 10 min at 4 °C. Protein concentration was determined using a BCA protein assay kit (P0010, Beyotime Biotechnology Co., Ltd.). The proteins were denatured at 100 °C for 10 min, separated by SDS-PAGE, and transferred to a PVDF membrane (Millipore, USA). The membrane was blocked with 5% skim milk powder for 2 h, then incubated overnight at 4 °C with primary antibodies against ERK1/2 (1:2000, Cell Signaling, USA), p-ERK1/2 (1:2000, Cell Signaling), and GAPDH

(1:20,000, Diagbio, China). After 16 h of washing, the membrane was incubated with corresponding secondary antibodies (1:3000) for 1 h. Protein signal intensity was detected and analyzed using ImageJ software for relative protein expression.

Statistical analysis

The results of each experiment were represented as means ± standard deviation (SD) with each experiment being carried out in triplicate and independently repeated at least 3 times. Differences between the groups were compared using one-way analysis of variance (ANOVA) with multiple comparisons made using Tukey's test and considered significant when $p < 0.05$ was reached.

RESULTS

High concentrations of taraxasterol are cytotoxic, and taraxasterol can alleviate the inhibition of HG on cell proliferation

When tested for drug toxicity at concentrations of 5 and 10 μg/ml, taraxasterol did not significantly inhibit MC3T3-E1 proliferation. However, a considerable suppression of proliferation was observed at 15 μg/ml ($p < 0.01$). Therefore, 10 μg/ml was selected for subsequent experiments (Fig. 1A). In the cell proliferation assay, the NC group showed significantly higher cell growth activity than the HG group on day 1 ($p < 0.001$). Between the NC and NC+ groups, there was no discernible difference ($p > 0.05$). Following the taraxasterol intervention, there was a significant difference in the cell growth activity between the HG+ and HG groups ($p < 0.001$). On day 4, once more, the NC group outperformed the HG group in terms of cell growth activity ($p < 0.001$). After being treated with taraxasterol, the cell growth activity of the NC+ group was less than that of the NC group ($p < 0.01$). On day 7, there was a significant difference in the cell growth activity between the NC and HG groups ($p < 0.001$). Following the treatment with taraxasterol, the cell growth activity of the HG+ group was considerably higher than that of the HG group ($p < 0.001$), while the activity of the NC+ group was less than that of the NC group ($p < 0.01$) (Fig. 1B).

Taraxasterol can promote the activation of alkaline phosphatase

Observations from the ALP activity assay indicate a non-significant decline in ALP activity under high glucose conditions on days 1 and 4 ($p > 0.05$). On the 7th day, nevertheless, ALP activity of the NC group considerably outpaced that of the HG group ($p < 0.001$). Following taraxasterol administration, there was a significant difference ($p < 0.01$) in the ALP activity between the HG+ and HG groups. In a similar vein, ALP activity was considerably greater in the NC+ group ($p < 0.05$) than in the NG group (Fig. 2).

Taraxasterol reduces OS under HG conditions

ROS staining showed that the fluorescence in the NC and NC+ groups was less intense and more dispersed. In contrast, under HG conditions, denser fluorescence areas were observed in HG group cells. Following taraxasterol intervention, the fluorescence area was reduced (Fig. 3A). The quantitative analysis in Image J did not reveal a statistically significant difference ($p > 0.05$) between the NC and NC+ groups. Under HG conditions, OS increased ($p < 0.001$), and taraxasterol intervention inhibited OS ($p < 0.001$) (Fig. 3B).

Taraxasterol enhances the clarity of actin structures and facilitates cell stretching under HG conditions

In the NC group, cells displayed a well-spread, clear polygonal shape with visible cytoskeleton structures and actin filaments. In the NC+ group, the internal structure of actin filaments was even more defined, although the polygonal shape was not as pronounced as in the NC group. The HG group exhibited poor cell spreading, and the actin filaments were obscured. Following taraxasterol treatment, cell spreading in the HG+ group improved, the cells became more elongated, and the clarity of actin filaments was also enhanced (Fig. 4A). According to Image J analysis, the cell spreading area was markedly reduced in the HG condition ($p < 0.01$) with no significant changes in the NC+ group ($p > 0.05$). The cell spreading area in the HG+ group increased following taraxasterol treatment ($p < 0.01$) (Fig. 4B).

Taraxasterol increases the expression of osteogenic genes, ALP and Runx2

The research found that relative to the NC group, the HG group exhibited decreased levels of ALP mRNA expression following the HG intervention ($p < 0.05$). In both groups, the levels of ALP mRNA significantly increased after taraxasterol therapy ($p < 0.01$) (Fig. 5A). Compared to the NC group, the HG group had reduced expression of Runx2 mRNA ($p < 0.01$). After being treated with taraxasterol, the Runx2 mRNA expression levels in both the NC+ and HG+ groups significantly rose ($p < 0.05$) (Fig. 5B).

Taraxasterol modulates the activation of ERK and p-ERK

Fig. 6A displays the Western blot results. ERK expression was significantly elevated in the HG group subjected to high glucose compared to the NC group ($p < 0.05$). Following taraxasterol treatment, ERK levels in the HG group exhibited a decreasing trend, though this was not statistically significant ($p > 0.05$). No significant differences in ERK expression were found between the NC and NC+ groups ($p > 0.05$) (Fig. 6B). For p-ERK, no significant statistical difference was observed between the NC and NC+ groups. High glucose

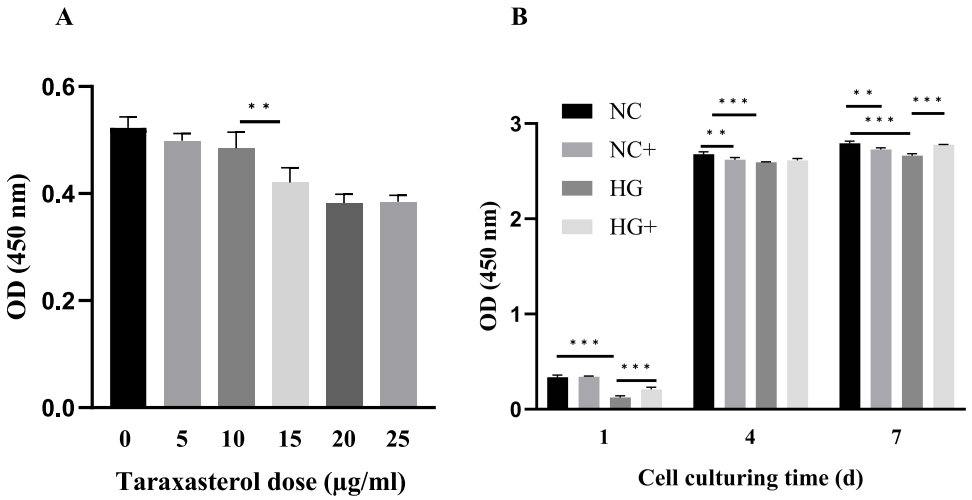

Fig. 1 Taraxasterol toxic to cells when the concentration is greater than 10 μg/ml and promoting the proliferation of cells under conditions of high glucose. (A) Taraxasterol intervention in MC3T3-E1 cells to detect drug toxicity. Following a 24-h exposure to different dosages of taraxasterol, the CCK-8 test was used to measure the proliferation of MC3T3-E1 cells. (B) Different batches of MC3T3-E1 cells cultured for 1, 4, and 7 days. The CCK-8 test was used to measure cell proliferation. The mean±SD, $n = 3$, **, $p < 0.01$; ***, $p < 0.001$ are the shown results.

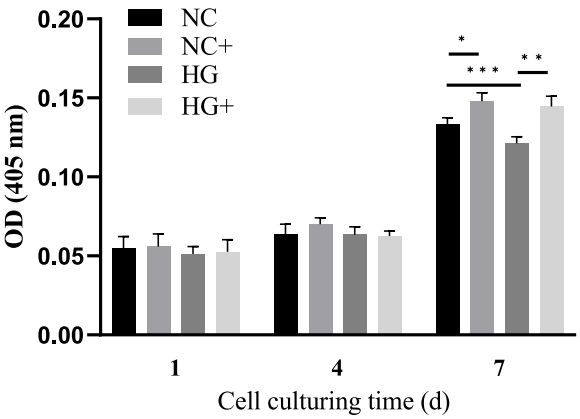

Fig. 2 Taraxasterol and high-glucose impact on MC3T3-E1 ALP activity. After growing different groups of MC3T3-E1 cells for 1, 4, and 7 days, the activity of ALP was assessed using an ALP kit. The results are expressed as mean ± SD; $n = 3$; *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

stimulation led to a substantial increase in p-ERK levels in the HG group ($p < 0.01$). Taraxasterol significantly reduced p-ERK expression in the HG+ group ($p < 0.01$) (Fig. 6C).

DISCUSSION

Diabetes is a prevalent disease in modern society with an increasing incidence in recent years. Elevated blood glucose levels in diabetic patients adversely affect various cell types. Prolonged exposure to HG in the body fluids of diabetic patients can disrupt osteoblast distribution. Research has demonstrated that MC3T3-E1 cells are susceptible to damage and apoptosis when exposed to HG levels [3]. Diabetic osteoporosis develops due to OS and other diabetes-related variables [12]. Several illness models have shown that taraxasterol is effective as a treatment, including arthritis, gastritis, enteritis, and liver injury. Furthermore, it decreases MPO levels while increasing SOD and CAT activity, which leads to a decrease in OS in rats suffering from sepsis-induced ARDS [13]. By blocking the activation of ERK and c-Jun NH2-terminal kinase (JNK) via the MAPK pathway, taraxasterol protects against acute kidney injury (AKI) caused by ischemia/reperfusion injury (IRI) [14]. In osteoblast-like MC3T3-E1 cells, high glucose conditions activate the ERK pathway, leading to alterations in osteogenic gene expression and resulting in osteoblast dysfunction [15]. Research has shown that taraxasterol blocks the MAPK pathway from getting activated. It is not yet known, however, how it affects osteoblasts when exposed to HG levels. Discovering how taraxasterol protects bones in HG conditions is the primary goal of this research.

The test was initially performed with various concentrations of taraxasterol over the culture of MC3T3-E1 cells to determine drug toxicity. According to the CCK-8 test results, cell proliferation was inhibited by taraxasterol of a concentration higher than 10 μg/ml. Hence, the concentration at 10 μg/ml was used in further experiments. It was reported that at glucose over 25.0 mmol/l, the biological characteristics of MC3T3-E1 cells were suppressed [3]. Thus, cells were

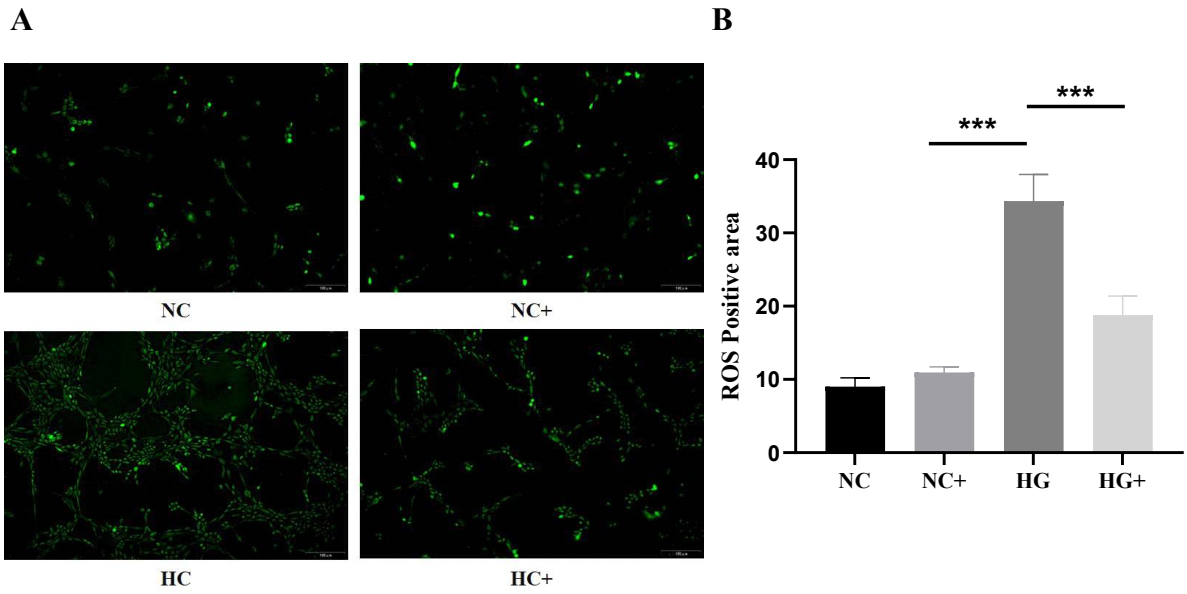

Fig. 3 In MC3T3-E1 cells, taraxasterol decreasing ROS generation when glucose levels are high. (A) The DCFH-DA probe used to detect ROS, which is displayed as green fluorescence (size bar = 100 μm). (B) Fluorescence staining area quantitative analysis with Image J software ($n = 3$). Results are all displayed as mean ± SD. ***, $p < 0.001$.

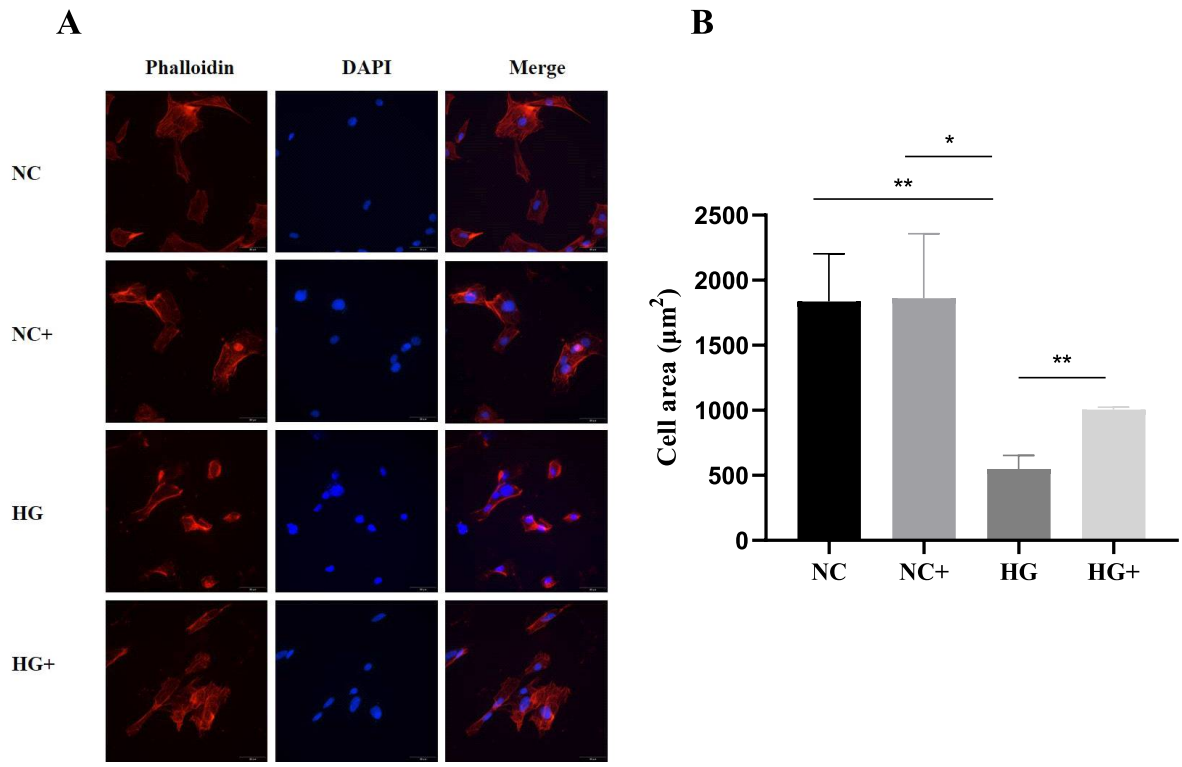

Fig. 4 Taraxasterol improving MC3T3-E1 cell spreading area and actin dynamics when exposed to HG. (A) The cell cytoskeleton and nucleus brought to light by staining the cells with Rhodamine-Phalloidin and DAPI, respectively (size bar = 50 μm). Rhodamine-Phalloidin staining is indicated by the red fluorescence, while DAPI staining is indicated by the blue fluorescence. (B) Quantitative analysis of the cell spreading area conducted using Image J software ($n = 3$). All results are presented as mean ± SD. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

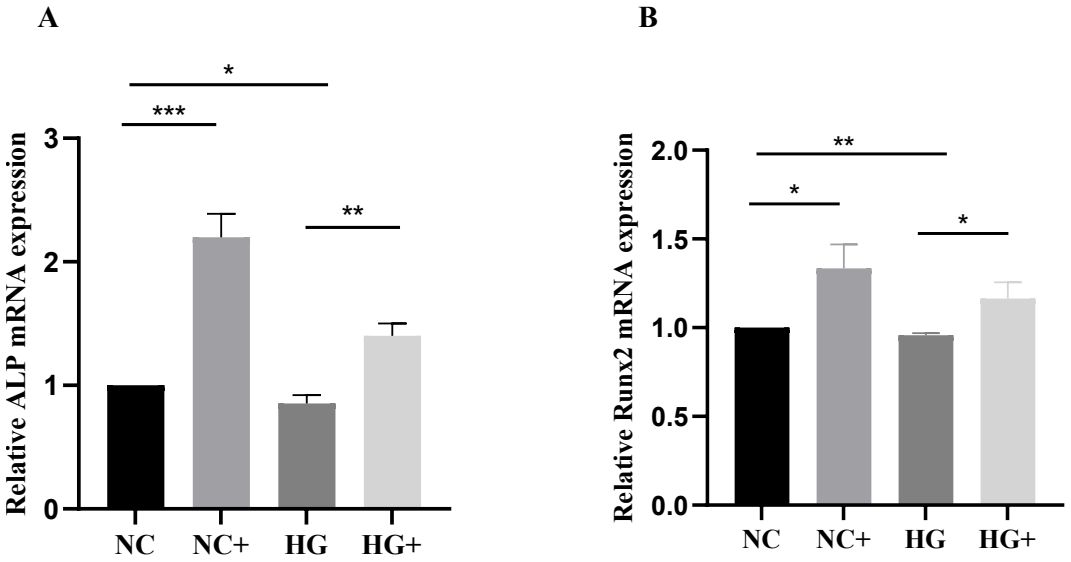

Fig. 5 ALP and Runx2 upregulated by taraxasterol. (A) MC3T3-E1 cells subjected to HG and taraxasterol treatment to quantitatively evaluate ALP expression using real-time PCR. (B) Runx2 expression assessed using real-time PCR in the same experimental setup. All results are presented as mean ± SD, $n = 3$. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

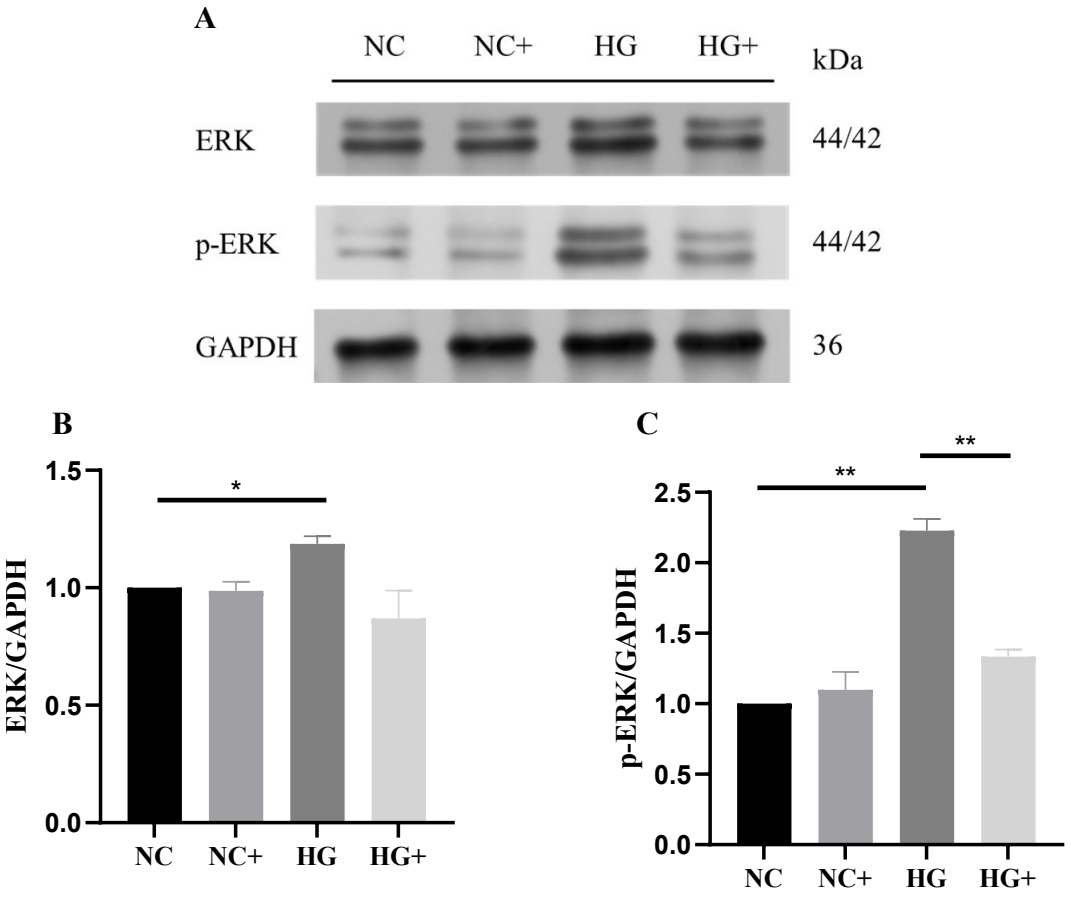

Fig. 6 Effects of taraxasterol on ERK and p-ERK protein expression under HG conditions. (A) Western blot analysis used to assess ERK and p-ERK protein expression following HG induction and taraxasterol intervention. (B and C) Quantitative analysis of ERK and p-ERK protein levels, respectively. All results are shown as mean ± SD, $n = 3$. *, $p < 0.05$; **, $p < 0.01$.

cultured in α-MEM medium with glucose concentrations of 5.5 mmol/l (NC group) and 25.0 mmol/l (HG group). Based on the presence of taraxasterol, groups were designated as NC, NC+, HG, and HG+. The CCK-8 proliferation assay revealed that high glucose significantly inhibited cell proliferation on day 1, aligning with the findings from Zhang et al. Taraxasterol treatment markedly increased cell proliferation in the HG+ group compared to the HG group, indicating that taraxasterol enhances MC3T3-E1 cell proliferation under HG conditions in a short timeframe. On days 4 and 7, NC group proliferation remained significantly higher than that of the HG group. However, after taraxasterol treatment, the NC+ group showed lower proliferation activity than the NC group, while proliferation of the HG+ group was significantly higher than that of the HG group on day 7. These results suggest that HG inhibits cell proliferation, an effect mitigated by taraxasterol. Nonetheless, prolonged taraxasterol exposure may induce toxicity and inhibit proliferation in normal glucose environments. Further investigation into the optimal duration of taraxasterol treatment and its associated toxicity is warranted.

Alkaline phosphatase is one of the earliest acting proteases in bone calcium formation [16] and serves as an indicator of osteoblast differentiation. Yang et al observed that ALP activity was inhibited in MC3T3-E1 cells after 48 h of HG exposure [17]. This study found that ALP activity in the HG group was marginally reduced on days 1 and 4, although these results were not statistically significant. By the 7 days, the ALP activity of the HG group had dropped significantly. Following delivery, taraxasterol increased ALP activity in the NC+ and HG+ groups relative to the control group, demonstrating that it facilitated the early differentiation of MC3T3-E1 cells. The trend observed in our results is consistent with the findings by Yang et al, although there is a discrepancy in the timing, which might be attributed to the different ALP assay kits used in the experiments. Additionally, the lack of significant differences in ALP activity between groups in the short term (1 day and 4 days) could be related to the varying maturity levels of the osteoblasts [18]. Further investigations are needed to clarify the temporal aspects of ALP activity in response to high glucose and taraxasterol intervention as well as to understand the underlying mechanisms of taraxasterol protective effects on osteoblasts under hyperglycemic conditions.

As a primary indicator of OS, ROS overexpression is important to note [19]. If ROS are not effectively cleared under oxidative stress conditions, they can cause damage to cells and tissues [20]. Research has demonstrated that MC3T3-E1 cells undergo OS when exposed to HG levels, which in turn induces ROS expression [12, 21]. This study reaffirms that an HG environment increases ROS expression, as shown by active oxygen analysis. Image J analysis revealed that with post-taraxasterol intervention, the ROS staining area was markedly reduced. Accordingly, even under HG circumstances, taraxasterol reduces OS in MC3T3-E1 cells. Furthermore, MC3T3-E1 cell adhesion, motility, proliferation, and differentiation are all significantly impacted by the actin cytoskeleton [22]. This experiment revealed that HG conditions caused the actin filaments in MC3T3-E1 cells to become less distinct and significantly reduced cell spreading, as evidenced by phalloidin staining. Following taraxasterol treatment, both the cell spreading area and the clarity of actin movement improved, indicating that taraxasterol enhances the early adhesion and extension of MC3T3-E1 cells under HG conditions.

Osteoblast development is intricately regulated by various extracellular and intracellular factors. ALP, an enzyme associated with bone metabolism and osteoblast calcification, increases its activity during bone formation, serving as a marker for osteoblast differentiation [23]. The Runt-related transcription factor (Runx) family, especially Runx2, plays a pivotal role in regulating cell differentiation and advancing the cell cycle [24]. Runx2 is particularly crucial for promoting osteoblast differentiation and bone mineralization [25]. An earlier study found that MC3T3-E1 cells under HG conditions reduced ALP and Runx2 mRNA expression [26, 27]. Consistent with these results, our experiment found the mRNA expression of both Runx2 and ALP to be decreased under HG conditions. In another study, Hu et al found that taraxasterol treatment upregulated the expression of ALP and Runx2 genes in osteoblasts [11]. Similarly, our research observed an increase in ALP mRNA expression following taraxasterol intervention, which was consistent with the elevated ALP activity detected. This suggests that taraxasterol enhances ALP activity by upregulating its gene expression. Furthermore, taraxasterol was shown to increase Runx2 expression in both the NC+ and HG+ groups. Research suggests that Runx2 directly targets the ALP gene and is essential for ALP activation [28]. The correlation between ALP and Runx2 expression in this experiment suggests that Runx2 may regulate ALP activity. Integrating our findings from cell proliferation assays and ALP activity measurements, we concluded that taraxasterol promotes the differentiation of MC3T3-E1 cells by modulating osteogenic gene expression. However, the observed inhibition of cell proliferation in the NC group treated with taraxasterol might be attributed to the specific drug concentration used or a slower proliferation rate during the differentiation process. Further research is necessary to explore the optimal concentration and duration of taraxasterol treatment to maximize its beneficial effects while minimizing potential cytotoxicity.

ERK 1/2, members of the MAPK family, transmit extracellular signals into the cell, thereby regulating cell proliferation, differentiation, and apoptosis

[29, 30]. Research has revealed that osteoblasts are more likely to undergo ERK phosphorylation in environments with HG levels [31]. Here, we found that MC3T3-E1 cell ERK and p-ERK expression levels rose in the HG condition but fell sharply in the HG+ group after taraxasterol treatment. According to research by Zhang et al, HG may stimulate ROS production in osteoblasts with ROS overexpression potentially linked to ERK pathway activation [7]. Other researchers have confirmed that activation of the ERK pathway is associated with the development of cellular OS [32]. This study observed that under HG induction, ROS levels and ERK and p-ERK expression rose. Extensive research indicates that ERK is an inflammatory pathway, and inflammation can lead to OS. Our experimental results provide further evidence that the upregulation of ROS is potentially related to the activation of the ERK pathway. Therefore, it is plausible that taraxasterol reduces OS in MC3T3-E1 cells under HG conditions by inhibiting ERK pathway activation. In short, this result not only confirmed the achievements that taraxasterol has a positive effect on the biological features of the MC3T3-E1 cell under the HG state but also laid the foundation for further research in connection with the relationship between the ERK pathway and osteogenic genes. Moving forward, we plan to use ERK pathway inhibitors in subsequent experiments to conduct a more detailed investigation of OS mechanisms. We will also explore other potential pathways and mechanisms that taraxasterol may regulate. Additionally, we intend to develop animal models to generate more comprehensive experimental data to support the protective effects of taraxasterol on osteoblasts in HG conditions.

CONCLUSION

In summary, this study explored the effects of taraxasterol on MC3T3-E1 cells under high glucose conditions. The findings indicate that taraxasterol enhances the proliferation and differentiation of these cells while reducing OS. The promotion of cell differentiation is associated with the upregulation of osteogenic genes, ALP and Runx2. The reduction in oxidative stress may be linked to the suppression of the ERK pathway activated by high glucose.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at http://dx.doi.org/10.2306/scienceasia1513-1874.2024.081.

Acknowledgements: Thank you to my supervisor, Assoc. Prof. Dr. Ramizu Bin Shaari for providing guidance and feedback throughout this project. Thanks also go to Dr. Mohamad Arif Bin Awang Nawi, Dr. Akram Bin Hassan, and Dr. Caiyun Cui for the thoughtful comments and recommendations on this dissertation.

REFERENCES

1. Cole JB, Florez JC (2020) Genetics of diabetes mellitus and diabetes complications. *Nat Rev Nephrol* **16**, 377–390.
2. Medeiros C, Wallace JM (2022) High glucose-induced inhibition of osteoblast like *MC3T3-E1* differentiation promotes mitochondrial perturbations. *PLoS One* **17**, e0270001.
3. Zhang P, Liao J, Wang X, Feng Z (2023) High glucose promotes apoptosis and autophagy of MC3T3-E1 osteoblasts. *Arch Med Sci* **19**, 138–150.
4. Guo Y, Pan W, Liu S, Shen Z, Xu Y, Hu L (2020) ERK/MAPK signalling pathway and tumorigenesis (Review). *Exp Ther Med* **19**, 1997–2007.
5. Liu J, Liu Z, Lu M, Zhang Y (2023) The combination of linagliptin and metformin rescues bone loss in type 2 diabetic osteoporosis. *J Drug Target* **31**, 646–654.
6. Liu H, Bian W, Liu S, Huang K (2012) Selenium protects bone marrow stromal cells against hydrogen peroxide-induced inhibition of osteoblastic differentiation by suppressing oxidative stress and ERK signaling pathway. *Biol Trace Elem Res* **150**, 441–450.
7. Wei-Lin Zhang, Meng HZ, Yang RF, Yang MW, Sun GH, Liu JH, Shi PX, Liu F, Yang B (2016) Melatonin suppresses autophagy in type 2 diabetic osteoporosis. *Oncotarget* **7**, 52179–52194.
8. Sharma K, Zafar R (2015) Occurrence of taraxerol and taraxasterol in medicinal plants. *Pharmacogn Rev* **9**, 19–23.
9. Zhang Y, Shaari R Bin, Awang Nawi MA Bin, Hassan A Bin, Cui C (2024) Pharmacological action and research progress of Taraxasterol. *Curr Pharm Biotechnol* **25**, 1767–1777.
10. San Z, Fu Y, Li W, Zhou E, Li Y, Song X, Wang T, Tian Y, et al (2014) Protective effect of taraxasterol on acute lung injury induced by lipopolysaccharide in mice. *Int Immunopharmacol* **19**, 342–350.
11. Hu R-Y, Li B, Sun L, Xu F-B, Yang X-T, Luo R, Tian X-B, Zhao J-M (2018) Taraxasterol as a proliferation-enhancing agent to enhance osteogenic differentiation of rat osteoblastic cells. *Int J Clin Exp Med* **11**, 528–538.
12. Chen B, He Q, Yang J, Pan Z, Xiao J, Chen W, Chi W, Li M, et al (2023) Metformin suppresses oxidative stress induced by high glucose via activation of the Nrf2/HO-1 signaling pathway in type 2 diabetic osteoporosis. *Life Sci* **312**, 121092.
13. Bu C, Wang R, Wang Y, Lu B, He S, Zhao X (2022) Taraxasterol inhibits hyperactivation of macrophages to alleviate the sepsis-induced inflammatory response of ARDS rats. *Cell Biochem Biophys* **80**, 763–770.
14. Li C, Zheng Z, Xie Y, Zhu N, Bao J, Yu Q, Zhou Z, Liu J (2020) Protective effect of taraxasterol on ischemia/reperfusion-induced acute kidney injury via inhibition of oxidative stress, inflammation, and apoptosis. *Int Immunopharmacol* **89**, 107169.
15. Liang B, Shen X, Lan C, Lin Y, Li C, Zhong S, Yan S (2021) Glycolipid toxicity induces osteogenic dysfunction via the TLR4/S100B pathway. *Int Immunopharmacol* **97**, 107792.
16. Ren MS, Xie HH, Ding Y, Li ZH, Liu B (2023) Erxian decoction drug-containing serum promotes Mc3t3-e1 cell proliferation and osteogenic differentiation via regulating BK channel. *J Ethnopharmacol* **302**, 115887.

17. Yang J, Ma C, Zhang M (2019) High glucose inhibits osteogenic differentiation and proliferation of MC3T3 E1 cells by regulating P2X7. *Mol Med Rep* **20**, 5084–5090.
18. Boughammoura S, Zarka M, Messaoudi I, Solal MC (2021) Interactions between cadmium and zinc on gene expression pattern of differentiation markers in MC3T3-E1 cell line. *Xenobiotica* **51**, 1038–1046.
19. Kalous KS, Wynia-Smith SL, Smith BC (2021) Sirtuin Oxidative Post-translational Modifications. *Front Physiol* **12**, 763417.
20. Ahmad S, Mahmood R (2019) Mercury chloride toxicity in human erythrocytes: enhanced generation of ROS and RNS, hemoglobin oxidation, impaired antioxidant power, and inhibition of plasma membrane redox system. *Environ Sci Pollut R* **26**, 5645–5657.
21. Takanche JS, Kim JE, Han SH, Yi HK (2020) Effect of gomisin A on osteoblast differentiation in high glucose-mediated oxidative stress. *Phytomedicine* **66**, 153107.
22. Kazmers NH, Ma SA, Yoshida T, Stern PH (2009) Rho GTPase signaling and PTH 3-34, but not PTH 1-34, maintain the actin cytoskeleton and antagonize bisphosphonate effects in mouse osteoblastic MC3T3-E1 cells. *Bone* **45**, 52–60.
23. Yang J, Yu K, Liu D, Yang J, Tan L, Zhang D (2021) Irisin enhances osteogenic differentiation of mouse MC3T3 E1 cells via upregulating osteogenic genes. *Exp Ther Med* **21**, 580.
24. Mevel R, Draper JE, Lie-A-Ling M, Kouskoff V, Lacaud G (2019) RUNX transcription factors: Orchestrators of development. *Development (Cambridge)* **146**, dev148296.
25. Komori T (2017) Roles of Runx2 in skeletal development. *Adv Exp Med Biol* **962**, 83–93.
26. Zhang L, Zheng L, Li C, Wang Z, Li S, Xu L (2019) Sema3a as a novel therapeutic option for high glucose-suppressed osteogenic differentiation in diabetic osteopathy. *Front Endocrinol (Lausanne)* **10**, 00562.
27. Liu Z, Yang J (2020) Regulatory effect of uncarboxylated osteocalcin on osteogenic and adipogenic differentiation of mouse bone marrow mesenchymal stem cells under high-glucose conditions. *Chin J Tissue Eng Res* **24**, 2039–2046. [in Chinese]
28. Weng JJ, Su Y (2013) Nuclear matrix-targeting of the osteogenic factor Runx2 is essential for its recognition and activation of the alkaline phosphatase gene. *Biochim Biophys Acta Gen Subj* **1830**, 2839–2852.
29. Liu F, Yang X, Geng M, Huang M (2018) Targeting ERK, an Achilles' Heel of the MAPK pathway, in cancer therapy. *Acta Pharm Sin B* **8**, 552–562.
30. Tanimura S, Takeda K (2017) ERK signalling as a regulator of cell motility. *J Biochem* **162**, 145–154.
31. Wang W, Zhang X, Zheng J, Yang J (2010) High glucose stimulates adipogenic and inhibits osteogenic differentiation in MG-63 cells through cAMP/protein kinase A/extracellular signal-regulated kinase pathway. *Mol Cell Biochem* **338**, 115–122.
32. Mi Y, Xiao C, Du Q, Wu W, Qi G, Liu X (2016) Momordin Ic couples apoptosis with autophagy in human hepatoblastoma cancer cells by reactive oxygen species (ROS)-mediated PI3K/Akt and MAPK signaling pathways. *Free Radic Biol Med* **90**, 230–242.

Appendix A. Supplementary data

Table S1 The primers used in this experiment.

Gene	Forward primer	Reverse primer
ALP	5′-TGACTACCACTCGGGTGAACC-3′	5′-TGATATGCGATGTCCTTGCAG-3′
Runx-2	5′-CATCCCAGTATGAGAGTAGGTGT-3′	5′-GCTCAGATAGGAGGGGTAAGAC-3′
GAPDH	5′-GACTTCAACAGCAACTCCCAC-3′	5′-TCCACCACCCTGTTGCTGTA-3′

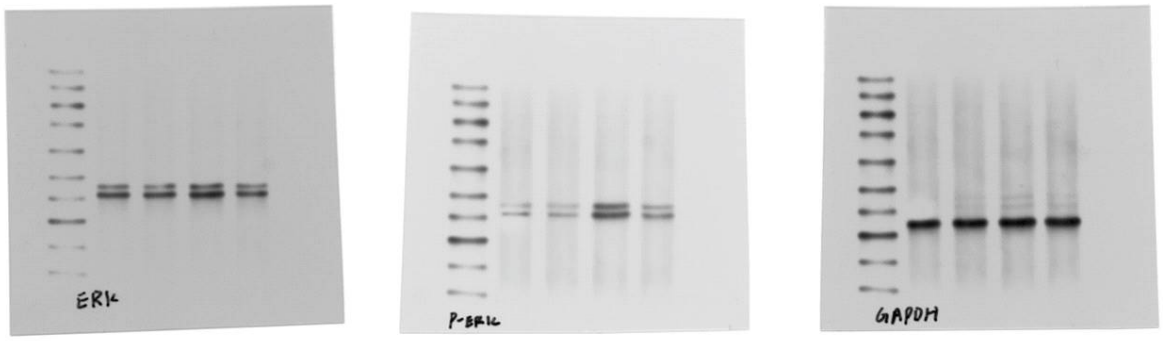

Fig. S1 Whole blots.